

SPLEEN PUNCTURE

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BY

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PREFACE

IN comparison with the first Swiss edition of 1947 the English edition contains new material and forty fresh figures

Recent hematological literature has been reviewed as completely as possible

I am grateful to many colleagues both Swiss and foreign who have supplied me with interesting spleen punctures

May the book in its present amplified form play a part in calling attention to the value of this method of investigation

I thank my chief Professor Löffler for his continuous help and interest Professor von Meyenburg for access to histological preparations and autopsy reports Professor Schinz and Professor Brunner for clinical records and Misses Ernst and Obrecht for evaluating so many splenograms Miss Meier has also assisted in the labour for which I am most grateful as I am to Mr Walch for the photo micrographs Special thanks are due to Mrs Bollinger Schudel for her excellent water colour drawings and to Dr Pinev for his careful and painstaking translation of this English edition

The publishers also have earned my gratitude for the care with which they have produced the book

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MEDICAL CLINIC OF THE UNIVERSITY
OF ZÜRICH SWITZERLAND

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(3) Puncture must not be performed in the presence of a recent septic splenomegaly or in the case of a painful spleen whether due to stretching of the capsule or to infarction

(4) Unconscious patients must never be punctured

(5) Rigid aseptic precautions must be taken

We are convinced that in time spleen puncture will become much more widely used because it is of such great value in the diagnosis of splenomegalies of clinically uncertain origin. In addition this method gives valuable information as to the condition of the spleen in various diseases and as a result of different therapeutic methods. Puncture of the sternal marrow, combined with puncture of glands and of the spleen often throws considerable light on the course and distribution of various diseases of the hemopoietic system.

The purpose of the present book is the discussion of the technique and the results of spleen puncture based on some 300 personal observations between the years 1939 and 1949 in the Zurich Clinic in the hope that this method will obtain a much wider acceptance. At the same time the literature of the subject is critically discussed and correlated with personal observations.

CONTENTS

	PAGE
PREFACE	v
INTRODUCTION	vii
PART I GENERAL	
HISTORICAL	1
TECHNIQUE	2
Site of Puncture—The Puncture Needle—Technique of Puncture —Marking of Cells	
CYTOGENETIC PROBLEMS	12
Monocytes—Plasma Cells	
CELLULAR STRUCTURE OF SPLEEN FLUID	16
Normal Spleen Cells	
RETICULO-ENDOTHELIAL ELEMENTS	17
Serosa Cells—Pulp Cells—Lymphoid Reticulum Cells—Macro phages—Tissue Mast Cells—Plasmacytoid Reticulum Cells	
LYMPHATIC SERIES	30
Lymphoblasts—Lymphocytes—Plasmoblasts	
IMMATURE MYELOID CELLS FROM HÆMOPHOETIC FOCI IN THE SPLEEN	34
MATURE GRANULOCYTES	35
THROMBOCYTES	35
CELLS OF LUNG ALVEOLI	36
Structure	
PATHOLOGICAL CELLS IN SPLEEN PUNCTURE	37
MITOSES IN SPLEEN PUNCTURES	37
Mitoses in Reticulo-endothelial Cells—Pulp Cells and Tissue Mast Cells—Lymphoblasts—Plasmoblasts—Erythroblasts	
THE LYMPHOCYTES MITOSES METABOLISM LIFE SPAN AND FUNCTIONS	40
AMITOTIC CELL DIVISION	43
THE SPLENOGRAM	44
HYPERSPLENISM AND INHIBITION OF THE MARROW	50
Clinical Features—Ætiology—Occurrence of Hypersplenism	
PART II SPECIAL	
THE SPLENOGRAM IN SPLENOMEGALIES	53

	PAGE
NON INFLAMMATORY HYPERPLASIAS OF THE SPLEEN	53
CIRRHOSIS OF THE LIVER	53
Laennec's Cirrhosis—Splenomegalic Cirrhosis—Wilson's Disease—Hemochromatosis	
THROMBOSIS OF THE SPLENIC AND PORTAL VEINS	58
SPLENOMEGALY WITH ECTOPIC FOCI OF BLOOD FORMATION	60
Mixed Myelocytic Erythroblastic Types—Compensatory Types—Chronic Inflammatory—Leukæmic—Predominantly Erythroblastic—Cases with Numerous Megakaryocytes	
OSTEOSCLEROTIC AND MYELOSCLEROTIC ANÆMIAS	64
HÆMOLYTIC ANÆMIAS	69
PERNICIOUS ANÆMIA	73
THROMBOCYTOPENIA WITH SPLENOMEGALY	75
SPLENOMEGALIES OF INFLAMMATORY ORIGIN	79
Acute and Subacute Splenomegalies—Neutrophile Reactions—Epidemic Hepatitis—Other Virus Infections—Q Fever—Lymphocytosis Acuta—Non Inflammatory Lymphatic Reactions	
CHRONIC INFLAMMATORY SPLENOMEGALIES	93
Characters of the Chronic Inflammatory Spleen Film—Sepsis Lenta—Still Chauffard Felty Disease—Felty Syndrome—Malaria	
GRANULOMATOUS CONDITIONS	103
Structure of Epithelioid and Langhans Giant Cells—Sarcoidosis—Syphilis—Morbus Bang—Mycoses of the Spleen	
HODGKIN'S DISEASE	113
NEOPLASTIC DISEASES OF THE SPLEEN	119
HÆMOBLASTOSES (LEUKÆMIAS)	119
CHRONIC LYMPHATIC LEUCOSIS	120
ACUTE LYMPHATIC LEUCOSIS	127
MYELOCYTIC LEUCOSIS (CHRONIC MYELOID LEUKÆMIA)	130
Differences in the Frequency and Distribution of Mitoses in the Spleen and Bone Marrow	138
The Effects of X Rays on the Spleen and Marrow	140
Changes in the Spleen and Sternal Marrow after Treatment with Arsenic	145
The Effects of Urethane on the Spleen and Marrow	
Pictures in the Leucoses	150
Chronic Lymphatic Leucosis Chronic Myelocytic Leucosis Structural Changes in Mitoses Prolonged Treatment with Urethane Acute Leucosis—Discussion and Summary	

CONTENTS

XI

PAGE

Nitrogen Mustard	165
Radio active Phosphorus	166
PARAMYELOBLASTIC LEUCOSIS	167
POLYCYTHÆMIA VERA	171
ERYTHROLEUCOSSES AND ERYTHROBLASTOSES	172
EOSINOPHILE BASOPHILE AND MEGAKARYOCYTE LEUCOSSES	175
PRIMARY MALIGNANT RETICULOSES AND RETICULO-ENDOTHELIOSES	176
SARCOMAS OF THE SPLEEN	189
Lymphosarcoma—Follicular Lymphoblastoma	
OTHER FORMS OF SARCOMAS	194
CARCINOMAS AND CYSTS	197
DEGENERATIVE CONDITIONS OF THE SPLEEN	198
Amyloidosis	
STORAGE DISEASES OF THE SPLEEN	198
Gaucher's Disease—Niemann Pick Disease	
SPLENOMEGALY OF UNCERTAIN ÆTIOLOGY	201
Splenomegaly with Intense Lipæmia—Splenomegaly with Lymphocytosis	
SUMMARY	204
EPILOGUE	210
BIBLIOGRAPHY	211
INDEX	227

PART I GENERAL

1 HISTORICAL

SPLEEN puncture was probably first performed by Vidal and his school for the diagnosis of typhoid fever towards the end of the last century when the bacilli were obtained in culture. For the same purpose Hayashihava performed 109 punctures (1905) without any complications. Later spleen puncture was performed in the Mediterranean countries for the diagnosis of Leishmaniasis (Kala Azar). The procedure was used for the same purpose in France by Nicolle (1909) Aravantinos (1916) Corona (1922) Giraud (1925) Benhamou (1934) and in Spain by Pittaluga (1935). The first clinical publication from Central Europe was that of Nagy (1924) to whom the credit should be given for introducing the method of puncture during deep inspiration. He pointed out that all the accidents previously observed (Wohlgemuth) were due to laceration of the capsule when spleen puncture was performed during expiration or in a mid way position because contraction of the diaphragm during puncture produced movement of the spleen in relation to the fixed needle. Nagy stresses the value of this method of examination for clinical diagnosis. Mele (1925) reached the same conclusion on the basis of 55 cases in Algiers. In pædiatrics Nassau (1926) employed spleen puncture for diagnosis and in one case of military tuberculosis was able to discover tubercle bacillus in films.

In Italy spleen puncture was used mainly for the diagnosis of Hodgkin's disease. Introzzi (1932) was probably the first to describe the typical Sternberg cells in films from spleen puncture—a finding which was later confirmed by Storti (1935). Gosio (1947) has carefully investigated the spleen in malaria. Spleen puncture was also used quite early for the diagnosis of Gaucher's disease (for literature see p. 198). All the publications so far mentioned have dealt with individual diseases and Weil (1934) together with Isch Wall and Perles were the first to undertake systematic investigation of the various forms of splenomegaly by spleen puncture. In Poland Tempka (1938) attempted to investigate the normal spleen by puncture of the organ during laparotomy. More recently Forconi (1939) has reported in detail on spleen puncture in erythroblastosis and there is a Spanish publication by Lopez (1939) dealing with splenomegaly in general. Heilmeyer and Schöner (1941) have also described the condition of the spleen in erythroblastosis. Spleen puncture in mononucleosis was described by us in 1941.

In the more recent text books on hæmatology spleen puncture does not receive much attention in fact only Heilmeyer (1942) lays

much stress on its diagnostic value and reports on his own observations in Hodgkin's disease, erythroblastosis and Gaucher's disease while Ferrata and Storti (1946) also attribute great value to it.

It is undoubtedly, going too far to suggest that spleen puncture is of greater diagnostic value than sternal puncture—a view which Weil supports; indeed, he writes as follows: 'Donc si a premiere vue les resultats (des myelogrammes) semblent satisfaisants une experience plus etendue montre que les classifications adoptees pour la rate n'ont pas ici (c'est a dire dans la ponction sternale) la purete et la precision que nous observons dans les splenogrammes.'

We certainly cannot agree with this view but it is undoubtedly a mistake to undervalue the significance of spleen puncture for diagnosis and we are convinced that in time this procedure will come to occupy a valued place in clinical investigation.

2 TECHNIQUE

ESTIMATION OF THE SIZE OF THE SPLEEN

(a) Palpation and Screening

It has already been mentioned that only definitely enlarged spleens should be punctured; in most cases it suffices to rely on palpation of the organ. If however the size of the spleen can only be recognised by percussion one should not attempt puncture unless very distinct enlargement is demonstrable. In all doubtful cases before deciding on spleen puncture it is advisable to examine the patient with the fluorescent screen when as a rule the organ can be seen lying between the bubble of gas in the stomach and the diaphragm where it appears as a dark oval shadow. If this is not demonstrable but if percussion has really aroused a suspicion that the spleen is enlarged one can try the following method: while the patient is being examined with the fluorescent screen he can be given 10 grammes of bicarbonate in a little water followed by a small amount of lemon squash; the gaseous distension of the stomach will then make the spleen easily visible.

(b) Adrenalin Test

In some cases further assistance in deciding whether a swelling in the left hypochondrium is really the spleen can be obtained by an intramuscular injection of 1 mg. of adrenalin. If within 15 or 20 minutes there is a distinct diminution in the area of mass there is then no doubt that this consists of the spleen (Gosio *et al.*). A negative adrenalin test is however not conclusive evidence that the mass is not composed of the spleen but it is well worth while examining the blood 15 or 30 minutes after the injection because even if no contraction of the spleen can be detected by palpation there is

often an emigration of immature cells from this organ (for literature see Hortling)

It is essential to realise that the presence of movement on respiration is not conclusive evidence that a mass consists of the spleen because even a hypernephroma or a pancreatic cyst may move in a similar manner

(c) X ray

An enlarged spleen is usually detectable on an ordinary chest film several workers have however attempted to make the organ more easily visible by injection of radio opaque substances which are stored in the reticulo endothelium At first Thorotrast was tried but was given up because its radio activity was dangerous Jentzer and Weyeneth (1944) have used Hepatoselectan which is a colloidal preparation of iodine which enables the spleen to be visualised very distinctly Gosio (1947) and others have carried out similar investigations

The use of such radio opaque substances is only of practical value when it is necessary to decide whether a palpable area of resistance in the left hypochondrium is really a spleen or whether it is due to some other tumour The fact that Hepatoselectan is stored only in the liver and spleen not in such masses as hypernephroma cysts sarcoma etc gives definite information In the majority of cases however percussion palpation and screening amply suffice

SITE OF PUNCTURE

Most authors have chosen the site for puncture in the area of the greatest definite dullness on percussion whether this happened to lie medial to the costal arch which involved puncture through the abdominal wall or laterally inserting the needle through the lowest intercostal space For the sake of safety we have limited ourselves to puncture the spleen in the ninth or tenth intercostal space a little more laterally or medially according to the position of the diaphragm on deep inspiration and according to the size of the area of splenic dullness Weil also seems to prefer this procedure

The topography of the spleen Fig 1 shows that puncture of the organ at the level of the ninth or tenth intercostal space in the position of complete inspiration will avoid all the adjacent organs The colon which lies antero laterally cannot be reached at this level and the stomach is also safeguarded by its medial position and by the interposition of the spleen The lower lobe of the left lung may extend down into the phrenico costal angle at the level of the tenth intercostal space but puncture of this is quite harmless because of the absence of any large vessels in the peripheral part of the lung Such penetration of the lung can usually be avoided if the lowest limit of pulmonary resonance during full inspiration is determined

by light percussion then the needle can be inserted 5 cm below this level. When the puncture is performed more in the medial part of the ninth or tenth intercostal spaces the lung is avoided because the lobe of the lung does not reach so far medially.

We regard puncture of the spleen through the abdominal wall as being definitely contra indicated even when palpation seems definitely to exclude the interposition of some other organ e.g. the omentum stomach, etc., between the abdominal wall and the spleen. There is no doubt that it is extremely difficult to be certain of this.

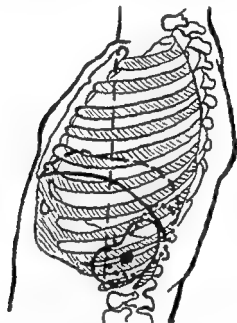


FIG. 1. The broken line shows the position of the spleen in expiration and the continuous line that in inspiration. Puncture is performed in the ninth or tenth intercostal space in the mid axillary line according to the area of maximum dullness and three or four fingers' breadth from the costal margin the whole procedure being carried out while the breath is held after a full inspiration.

especially in obese patients. Furthermore direct puncture of the spleen through the abdominal wall does not permit of such certain fixation of the needle which may be displaced by contraction of the abdominal muscles and so cause small laceration of the splenic capsule. Also direct puncture because it is done more medially and the spleen lies deeper increases the chance of confusion so that a greatly enlarged left lobe of the liver or a hypernephroma or a pancreatic cyst can be misleading. This is not likely to occur when careful percussion in the lateral region at the level of the ninth or tenth intercostal space fails to elicit dullness. But even using the intercostal route such surprises may occur and we ourselves

have inadvertently punctured a hypernephroma and also a pancreatic cyst. The main argument in favour of the intercostal method is the fact that the upper pole of every definitely enlarged spleen lies in the left lateral sub diaphragmatic space directly in contact with the ribs without the possible interposition of any other organ.

THE PUNCTURE NEEDLE

Weil used a needle 0.8-1 mm wide and 10 cm long with a stylet and a 2 or 5 c.c. record syringe. In our experience a rather wider and longer needle 1.2-2 mm in diameter and 12-15 cm in length with a ground in stylet is better. This increases the chance of aspirating small fragments of spleen tissue and the length of the needle usually suffices to prevent any of the material from entering

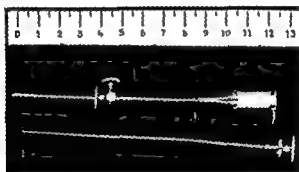


FIG. 2. Puncture needle with stylet and movable guard

the syringe—an event which usually spoils the result because the cells are disintegrated by being blown out of the syringe again.

The bevel of the needle should not be steep but it should be extremely sharp because most of the material is obtained during its insertion. If no special needle is available an ordinary lumbar puncture needle will serve. In all cases there should be a movable guard on the needle to prevent too deep a penetration. Both the needle and syringe must be sterilised and dry because even the smallest amount of water disturbs the normal cell structure.

The syringe should be a very well fitting 20 c.c. record syringe with which powerful aspiration is possible. Smaller syringes such as were used by Storti and Weil are not so satisfactory because of the lesser degree of negative pressure.

Spleen Puncture by the Harpoon Method

It seemed obvious that spleen puncture with a liver puncture needle of Roholm and Iversen or with Silvermann's needle might be tried so as to obtain definite pieces of spleen suitable for histo-

by light percussion then the needle can be inserted 5 cm below this level. When the puncture is performed more in the medial part of the ninth or tenth intercostal spaces the lung is avoided because the lobe of the lung does not reach so far medially.

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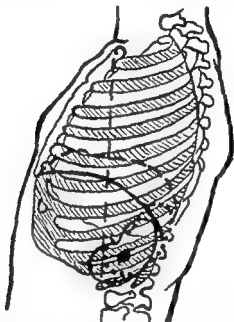


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and a 5 cm record syringe containing 4 c.c. of a local anæsthetic e.g. 2 per cent procaine. It is recommended that no risk should be taken with the sterility of the anæsthetic and that only ampoules should be used and not solutions sterilised in bottles because these are liable to contamination when used several times. First a cutaneous wheal should be made with the local anæsthetic and then the tissue should be infiltrated perpendicularly to the skin surface to a depth of 1-2 cm. the patient is then asked to breathe rapidly and superficially while the needle is slowly pushed on a millimetre at a time until there is a complaint of slight pain which usually indicates that the peritoneum has been reached. The needle is then pushed on 1 or 2 mm further until one can feel a scratching sensation as the point rubs against the spleen capsule. this depth should then be marked on the needle. If as is occasionally the case such vibration cannot be felt the depth of penetration to be marked on the needle is that at which the slight peritoneal pain was noticed. this is practically the same distance as the surface of the spleen. *This method permits exact measuring of the depth of puncture required to reach the splenic capsule.* Thus if we add 2 cm to this depth it enables us to fix at the same time the depth of the puncture in the spleen tissue itself and to limit it to 2 cm.

This modification of the technique of spleen puncture which we were the first to describe *permits a limitation of the penetration of the spleen to a minimum and thus prevents damage to any of the larger vessels.* The main vessels of the spleen enter at the hilum and end as a fine mesh work in the sub capsular region on the lateral aspect i.e. the site that is punctured. In this way there is no danger of penetrating the larger vessels at the hilum even if the spleen is not greatly enlarged and is of the flat type. Slow and careful introduction of the needle used for local anæsthesia rarely fails to demonstrate the distance of the spleen from the surface. If however as a result of extensive adhesions and a large almost immobile spleen it is not possible to determine the depth one must carefully penetrate to a depth of not more than 5-7 cm according to the degree of adiposity.

Having inæsthetised the area and decided on the exact depth of penetration the spleen puncture itself can be commenced. The guard on the needle should be fixed to 1.5-2 cm beyond the depth of the capsule and the needle should be pushed through the same spot between the ribs then the stylet is removed and the record syringe attached. The patient is now told to take a deep breath and then to close his mouth and pinch his nose between two fingers while the spleen is rapidly punctured. As soon as the guard prevents further penetration one aspirates quickly but strongly once or twice. Before the needle together with the attached syringe is withdrawn it is essential to release the negative pressure in the syringe by per

logical examination We would however warn anyone against the attempt because by this method of tearing a cellular piece of tissue out of the spleen far too many blood vessels are opened and as the needle punctures more deeply, there is the possibility of opening large vessels and so leading to severe bleeding In any case the examination of such larger pieces of tissue seems to be unnecessary in the majority of cases because it is particularly in the spleen that the structural characters of the cells and their quantitative distribution are best seen in films which certainly permit finer detail to be seen than do histological sections in which the cells are shrunk by fixation This applies particularly to the difference between lympho sarcoma and lymphatic leukemia, etc In the liver the conditions are very different because the histological structure rather than the cellular composition is the important feature If however the histology of the spleen is to be investigated it is best to use a needle of 3 mm in thickness and not to penetrate the organ more than 1 cm The technique of histological examination of such fragments is described on p 10

Technique of Puncture

Before undertaking a puncture, the bleeding time (Duke's method) and the coagulation time (Lee White's method) should be estimated the former being more important If this is considerably prolonged puncture should not be performed If the bleeding time is normal but the coagulation time slightly prolonged, puncture can usually be performed without danger If both values are increased it is better to avoid performing a puncture Slight diminution of platelets and of prothrombin are usually of no significance if the bleeding time is normal

Before proceeding with the puncture one must obtain a clear picture of the position of the spleen using most careful percussion and palpation The patient should lie flat on his back with no pillows under the head Puncture of the spleen with the patient in the lateral position is not to be recommended because the spleen then falls towards the middle line One should first percuss the spleen with the diaphragm in the middle position and then tell the patient to take a deep breath The exact upper limit of dullness should be marked on the skin and if light percussion is used this corresponds with the lower limit of the lung The puncture should be made at least 6 or 7 cm below this point in an area of complete dullness almost invariably this point lies 4 or 5 cm lateral to the costal margin in the mid axillary line in the ninth or tenth intercostal space

The area is carefully disinfected with merthiolate or iodine either of which can be used for indicating the point of puncture by a cross For local anaesthesia we use a fine needle about 10 cm long

Negative Punctures

When the area of splenic dullness is definitely increased puncture rarely fails to obtain some material. If however the organ is very hard as after intensive X ray treatment for Hodgkin's disease nothing may enter the needle.

If in spite of dullness two punctures prove abortive X ray screening should be done to prove that the spleen really is enlarged. If it is a puncture a little to one side of the original one may be successful.

After treatment and Possible Complications

Weil keeps his patients lying on their left side with an ice bag on the hypochondrium for 12 hours. As the puncture is so superficial when our technique is used we do not employ these precautions. We have been able to ascertain that punctures performed 1 or 2 days before death cannot be recognised at autopsy and that there is no trace of blood in the peritoneum.

Spleen fluid clots more rapidly than blood probably because of the very numerous platelets in the pulp and the liberation of thrombokinas from the damaged tissue cells. And as our puncture only extends into the sub capsular tissue there is probably reflex contraction of the vessels which in this region are all small. For this reason we do not advocate post operative injection of adrenalin.

As Nagy and Weil emphasise the few published examples of bleeding from spleen punctures were probably due to laceration of the capsule by puncturing the organ in the position of expiration or by puncturing a soft septic spleen or by failing to recognise the presence of a hemorrhagic disease.

The irritation of the puncture may cause a reflex contraction of the diaphragm and if the operation is not performed during full inspiration the spleen may be lacerated by movement against the needle. Hence the method here advocated should be carefully adhered to and in more than 300 spleen punctures we have never seen any signs of bleeding.

After the operation the patient should lie flat on his back for an hour but no ice bag is needed. He can then have a meal and remain in bed for a further 6 hours. The only complaint that may be made is of a slight stitch on inspiration which may be referred to the left shoulder. We then use a Dilaudid suppository and have always found that there is no further trouble. Out of 300 patients 8 complained of slight stitch lasting for 2 or 3 days but in all other cases only very slight discomfort was mentioned. The operation itself should be completely painless.

With proper precautions infection does not occur. If however a spleen abscess is suspected puncture should only be performed extraperitoneally during a laparotomy.

mitting the plunger to go back slowly - If this is not done negative pressure may bring blood or local anæsthetic into the syringe which makes the films useless. For the same reason no further aspiration must be performed while the needle is being withdrawn. The patient must hold his breath in the inspiratory position during the whole procedure and may only breathe again when the needle has been withdrawn. It is essential to pay particular attention to this and to practice the whole procedure apart from the puncture itself with the patient before the actual performance of the operation.

The inspiratory position is a basic requirement for the harmlessness of spleen puncture and for this reason the operation must not be performed on unconscious patients

If puncture is performed properly no blood enters the syringe but a few drops of serous fluid and perhaps a few bits of tissue can be blown out of the needle. In splenic inflammation and in cases in which there is portal hypertension or chronic congestion of the spleen only brief aspiration should be performed for fear of drawing blood into the syringe. The smaller the amount of blood sucked out the more cells there will be found in the films and they will be in a better state of preservation so that a more accurate splenogram can be obtained.

Gosio recommends that no aspiration should be performed and that one should limit one's examination to the material that enters the needle during the puncture. Our own impression is that short aspiration does however supply more material—particularly from firm spleens.

Preparation of Films

The material must be spread rapidly because spleen fluid coagulates even more quickly than marrow from sternal puncture. As soon as we have withdrawn the needle it is taken off the syringe, which is filled with air and re-attached to the needle then the contents are blown out on to one or more slides. Excess of blood can usually be removed with filter paper but this should not be necessary with good technique. Then with the edge of a suitable slide films are made of one or two drops of the puncture fluid. Of course if only one or two drops are obtained it is as well not to try to make more than one film. The spreading must be done without pressure and requires a certain amount of practice which can be obtained by making films from gland or sternal puncture.

If there are some fragments of tissue in the needle the histological method suggested by Rohr for sternal marrow can be used viz fixation and embedding in paraffin (see p. 10) obviously this combined cytological and histological method has much to recommend it.

states small collections of pigmented cells may be recognisable. These appearances are not constant and are much less important than the microscopic ones.

As a rule one can dispense with a detailed qualitative determination of the splenogram. Those given in this book are mainly intended as standards of comparison for other workers.

A detailed differential count is useful in some conditions such as aleukæmic lymphadenosis in which an increased percentage of lymphocytes is diagnostic. The splenogram is also useful in some cases of chronic myeloid leukaemia and cases of osteosclerotic and myelofibrotic anemia. The qualitative relationships of the various cells and the degree of maturity are often of prognostic significance in leukaemia. Apart from this the detailed splenogram is mainly of academic interest.

Low magnification ($\times 300$) should be used for a preliminary examination but in order to avoid glare it should be covered with a thin layer of cedar oil. At least two films should be thoroughly examined using the oil immersion lens for any peculiar cells. The best parts of the films should be fully examined with the highest magnifications. It is only in this way that scanty elements such as the Sternberg cells of Hodgkin's disease are likely to be detected.

The aspirated spleen tissue is derived from the red pulp, well from the follicles so that the different types of cells are not evenly distributed in the films. The cells from the pulp are found more evenly spread than those from the follicles which tend to occur in streaks (Fig. 25). Certainly marrow and gland films spread more evenly than does spleen juice. Of course experience helps a great deal. For instance if there is much blood spleen cells will be more widely distributed but will be found mostly at the edges of the films. This is a sound reason for not trying to obtain too much spleen juice: one or two quick aspirations suffice to obtain enough material in the needle. Even so a good deal of blood may be obtained then it may take time to ascertain whether any spleen material is present. If pulp cells or macrophages are seen there can be no doubt of the success of the puncture.

Marking of Cells

It is often useful to mark the position of cells so that they can be found again. The following is a simple method of doing this. A film is firmly clipped on the movable stage and the desired spot is examined with the oil immersion objective. This is then raised and the oil on the film is carefully removed but a small amount is left on the aperture of the objective. The tube is now racked down until the objective touches the preparation. A small ring of oil will be seen when the objective is raised and the spot can be marked on the back of the slide by a small ink circle.

For anatomy and physiology of the spleen refer to the literature on the subject.

A young man developed signs of generalised sepsis after a panaritium with great splenomegaly but repeated blood cultures were negative and chemotherapy proved useless. A spleen abscess was suspected and an operation was decided on. The spleen was surrounded by dense adhesions and puncture revealed staphylococcal pus. Recovery followed drainage of the splenic abscess.

Recent infarction is a contra indication to spleen puncture (Stubenrauch) because the friable tissue easily tears. Spleens that are tender on palpation must also not be punctured because the tenderness is clear evidence that the capsule is tense.

Staining Methods

Air dried films are stained by the May Grunwald Giemsa method allowing the Giemsa solution to act for 15–20 minutes instead of the usual 10 minutes. A few films should be retained for special staining e.g. Sudan if lipoid storage cells are present or Prussian blue staining if iron containing pigments are suspected (hæmo siderosis etc).

Histological Methods

If enough tissue is obtained it is advisable to embed one or more fragments for histological examination. We use the method suggested for sternal marrow by Rohr.

Fix in sublimated formol (saturated solution of sublimate 400 40 per cent formalin 100 glacial acetic acid 20) for 1 to 2 hours.

Wash in running water 1–2 hours.

70 per cent alcohol 12–24 hours or longer.

96 per cent alcohol about 12 hours.

Change of 96 per cent alcohol 12 hours.

Absolute alcohol 2–3 hours.

Chloroform $\frac{1}{2}$ –1 hour.

If the material is bloody use Carnoy's solution (absolute alcohol 6 chloroform 3 glacial acetic acid 1) instead of 96 per cent alcohol.

Embed oven at 60°C—soft paraffin (46°–48°) 2–3 hours, then hard paraffin (56°) 2–3 hours. Cool in water and block.

Sections 2–3 microns thick are mounted and placed in the oven (60°) for 1–2 hours. After cooling 10 minutes in Xylol then Xylol and alcohol equal parts followed by absolute alcohol I and II and then 70 per cent alcohol. Wash in water. Remove sublimate by placing in Lugol's iodine for 10 minutes then wash for $\frac{1}{2}$ –3 hours. Stain with hæmatoxylin and eosin or Romanowsky.

Examination of Films

As a rule little can be learned from the naked eye appearances but in leukæmic and lymphosarcomatous spleens the puncture fluid may be greyish and mixed with little blood. In hæmolytic

plasmacytoid reticulum cells and lymphatic plasma or Turk cells. The purely morphological differences between these two groups which will be discussed later are probably not alone sufficient to justify the subdivision but the great functional difference supports such a distinction.

Rohr and many other investigators (Nordensson, Schilling, Heilmeyer, etc.) believe that the plasma cells found in the blood arise from the plasmacytoid reticulum cells of the bone marrow and that they are therefore not as was previously believed derived from the lymphatic system (Maximow, Turk, Pappenheim, Naegeli). Clinically there is an increase of plasma cells in the blood in a variety of diseases especially in German measles and in infective hepatitis. We have undertaken a systematic investigation of the relationship of these elements in the sternal marrow and in gland puncture of cases of German measles and have been able to demonstrate that the plasma cells found in the blood certainly are not formed in the bone marrow but in the enlarged lymphatic glands (Moeschlin 2). In gland punctures of these cases one can find every transition from immature cells probably derived from the reticulo-endothelium to typical young and also mature plasma cells such as are found in the blood (Fig. 21) whereas in the marrow there is no increase of plasmacytoid reticulum cells or of the younger elements of the plasma cell series. We have called the parent of the plasma cells in lymphatic glands the plasmoblast (Fig. 11 = 21 a) which is quite different from the lymphoblast. This special type of cell only occurs in lymphatic tissue and our investigation suggests that it is probably directly derived from the large lymphatic reticulum cell which occurs in the germ centres.

These plasmoblasts are distinctly seen in histological preparations and the presence of such large cells with large vesicular nuclei was observed in the glands in cases of German measles by earlier investigators (DeBenedetti). These plasmoblasts with their large delicate nuclei are not found in the myeloid marrow even when there is considerable hyperplasia of the plasmacytoid reticulum cells e.g. in pneumonia. Even so our view has been controverted by some writers (Dubois, Ferrière, Heilmeyer, etc.) who hold to the opinion that the plasmacytoid reticulum cells and the plasma cells are morphologically and functionally one. On the contrary Landolt carried out an investigation on infective hepatitis and by sternal puncture was able to show that the plasma cells found in the blood were not formed in the marrow confirming our own investigations. Our own opinion based on spleen puncture in hepatitis (described later) was that the lymphatic tissue of that organ must be regarded as the site of formation of the plasma cells. Ludin who performed gland punctures agrees with the above view and Gormsen also recognises two separate series of plasma cells.

3 CYTOGENETIC PROBLEMS

Our classification of blood cells follows that of Naegeli who regarded the myeloblast as being the differentiated stem cell of the granulocytes. We have not differentiated reticulo endothelial cells into hæmohistioblasts fibroblasts etc. agreeing with Rohr that bias as to their genetic and functional state must be avoided. It is best to name the various reticulo endothelial elements on purely structural grounds.

Naegeli's views are opposed by the Italian school (of Ferrata) with whom Kienle has joined forces.

Ferrata derives the erythropoietic granulopoietic and thrombopoietic series from the hæmocytoblast which is structurally identical with the non granular myeloblast of Naegeli while the Italian school refer to those cells that contain fine azurophile granules (promyelocytes of Naegeli) as myeloblasts. They use the term hæmohistioblast to refer to a cell that to some extent at least corresponds with the parent of all blood cells and which in German literature is usually known as the Ferrata cell. It is probably no longer advisable to use this term because some of the elements known as hæmohistioblasts are non granular while others contain some granules and can therefore scarcely be given the status of stem cells. Naegeli and Rohr have pointed out that many of these so called Ferrata cells are artefacts and are really crushed premyelocytes or damaged leukæmic cells. The illustrations of more recent Italian hæmatologists (Fieschi and Storti) indicate that the non granular forms are identical with the lymphoid reticulum cells of Rohr while the pictures of granular hæmohistioblasts given by Kienle are by no means convincing. On the whole therefore we adhere to the views of Naegeli admitting however that the inferences as to cyto genesis are scientifically vulnerable and will doubtless require modification in future. Certainly our views of the genesis of monocytes and plasma cells differ from earlier opinions.

Monocytes

Naegeli regarded the monocytes as being purely of myeloid origin whereas Schilling held that they are of endothelial origin. Probably the truth lies midway between these views and we share Rohr's opinion that there are both myeloid and lymphatic monocytes (e.g. in some cases of lymphatic reaction) and also that some arise directly from the reticulo endothelium as in some infective states (reticular monocytes histiomonocytes) (see Fig 16 III A). We have been unable to find any support for Wuhrmann's view that the monocytes play a part in the formation of proteins.

Plasma Cells

On the basis of earlier investigations we have already asserted that the plasmacytoid elements can be divided into two groups viz

and in the marrow but not in lymphatic tissue. Such new experimental results appear to confirm the view that there are functionally two types of plasma cells.

Investigations with the phase contrast microscope (Moeschlin 23-24) showed that the cytoplasm of myeloma cells contained rather large dark granules which are not identical with the mitochondria and which do not stain with ordinary staining solutions (Fig 20 e). It is possible that these structures are really pathological high molecular globulins which are formed in these cells. It is interesting to note that we found similar dark globular structures in those active plasmacytoid reticulum cells (Fig 20 d) in inflammatory conditions (antibody formation) and also in small lymphoid reticulum cells (Fig 30 g). Fagraeus appears to have shown conclusively that the latter can be directly converted into the plasma cytoid type. Other pathological conditions in which there is a great increase of such small reticulum cells associated with striking changes in the blood proteins have also been recognised e.g. macroglobulinæmia of Waldenström. On the contrary the lymphatic plasma cells of German measles showed no dark granules in the examination by phase contrast (Fig 20 b c). At the present time our interpretation of these findings is to be regarded as nothing more than a working hypothesis which we hope to be able to prove by further observations.

We used to believe that the extramedullary myelomas which are perhaps partly derived from the lymphatic plasma cells do not produce any changes in the serum proteins (Jaeger). This is not confirmed by the latest investigations (Jequier, Doge, Heckner 1949).

Another differential feature is the possession of phagocytic powers by the plasmacytoid reticulum cells, a function which is not manifested by the plasma cells of the blood. The plasmacytoid reticulum cells do not only ingest fats and lipoids (see Fig 16 a) (Gormsen, Fagraeus etc.) but also hæmosiderin. We have seen this in cases of hæmolytic anæmia and also of aplastic anæmia in which repeated transfusions have been given (see Fig 17 d).

Dubois Ferrière (1943) demonstrated this phagocytic activity by injection of Indian ink into the marrow. We could however never detect any signs of phagocytic activity on the part of the plasmoblasts or of the blood plasma cells which we regard as being of lymphatic origin. We cannot agree to the opinion of Dubois Ferrière that the plasma cells are a state of lymphocytes or reticular cells characterised by the absorption of proteins. Especially the interesting examinations of Fagraeus who compared the number and the morphology of the plasma cells in the spleen with the titre of the antibodies in the blood contradict this opinion.

Plasmacytoid reticulum cells with typical reddish violet cytoplasm and relatively small nucleus are found in the spleen as are

In addition these investigations of the bone marrow and the lymphatic glands show that there are functional differences between the two series of cells. The plasmacytoid reticulum cells of the marrow appear to play a part in the formation or metabolism of certain globulins (Brass, Rohr, Fleischhacker, etc.). This certainly applies to the β and γ globulins which are found in myelomatosis and which may pass through the kidney (Bence Jones proteoses in the urine) and also into the cerebro spinal fluid (increased total protein, abnormal mastic and gold sol reactions) (see Moeschlin (9)).

The electrophoretic investigations of Wuhrmann and Wunderly etc. show that the abnormal proteins produced by the myeloma cells mainly belong to the group of γ globulins and are much more rarely of the β or α group. It has, therefore, been inferred that the plasmacytoid reticulum cells of the marrow, which are the parent cells of the pathological myeloma cells, are the source of at least a part of the serum globulin (Rohr, Fleischhacker, Klima, etc.). Rohr especially called attention to the parallel increase in the number of plasmacytoid reticulum cells and the level of globulin fraction in the blood in various diseases, e.g., chronic inflammatory diseases and cirrhosis of the liver.

Gormsen (1942) confirmed that this was approximately true while more recently he has been able to show that these plasmacytoid reticulum cells probably possess the function of producing anti bodies as was first suggested by Huebschmann (1913). Dougherty and White attributed this function to the lymphocytes (see p. 43) but Gormsen has shown in animal experiments, that the thymus which is so rich in lymphocytes does not form any anti bodies while extracts from the spleen (which contains many plasma cells) and also of the peri renal fat (which contains no lymphocytes but many plasma cells) contain much greater concentrations of anti bodies than do other bodies or the blood serum (Bjorneboe, Gormsen and Lundquist (1947)). Bing, Fagraeus and Thorell (1945) using the micro spectrographic method of Caspersson showed that this synthesis of anti bodies as well as the synthesis of cell protein occurs in association with ribose nucleotides. Fagraeus (1948) was able to extend Gormsen's work in so far as she found the highest concentration of anti bodies in the spleen of immunised animals when immature plasma cells were most numerous. Such anti bodies were very scanty in the lymph follicles of the spleen but plentiful in the red pulp. Kolouch (1947) observed that in sensitised rabbits the plasmacytoid reticulum cells decreased in size in the state of shock and changed into the small Marschalko type within four days.

These investigations justify the conclusion that the plasma cytoid reticulum cells play a great part in the formation of anti bodies and this process occurs mainly in the red pulp of the spleen.

increase of cells characteristic of infective reactions or the presence of pathognomonic elements such as epithelioid or Langhans giant cells or glandular fever cells etc. In leukemias and sarcomas films may contain a few characteristic cells or these may be so numerous as to dominate the picture.

The discussion will be divided into that of the cells found in the normal spleen and that of genuinely pathological forms but the latter will be dealt with later when individual diseases are discussed.

NORMAL SPLEEN CELLS

Strictly it is not true that the films are made from normal spleens because puncture is only done when there is distinct enlargement. Our knowledge of the normal spleen cells is therefore based on spleens removed at operation.

The cells can be divided into the following five groups —

- A Reticulo endothelial elements
- B Cells of the lymphatic series
- C Immature myeloid cells from hemopoietic foci
- D Mature granulocytes
- E Thrombocytes

A RETICULO ENDOTHELIAL ELEMENTS

Aschoff used the term reticulo-endothelium to designate the totality of cells that are characterised by the power of forming reticular fibrils and of picking up colloidal dyes after injection. In the spleen the reticulo endothelial apparatus is composed of the framework of the red pulp and the endothelial cells of the sinuses together with those cells that form the mesh like stroma and the lymph sinuses of the follicles. The latter is identical with the reticulo endothelial cells of lymphatic glands and for this reason spleen films will be found to contain those elements that are characteristic of the red pulp as well as those of the types found in gland punctures.

The nature of the germ-centre cells as Thiel and Downey call them is still disputed although transitions between them and lymphoblasts and large lymphocytes certainly do occur. We have therefore included them with the lymphatic cells although we are convinced of their reticulo endothelial nature and have used the name large lymphatic reticulum cells.

Forkner (1928) was one of the earliest to subdivide the reticulo endothelial cells into four groups — reticulum cells fibroblasts macrophages and tissue mast cells. Pavlovsky (1934) only recognises endothelial cells and macrophages. Tischendorf (1939) in his monograph on gland puncture speaks of undifferentiated endothelial reticulum cells fibroblasts macrophages and plasmacytoid reticulum cells while Stahel recognises young round cells sinus endothelium cells macrophages and tissue mast cells. Rohr was the first to subdivide and describe the

also the rather larger lymphatic plasma cells with a relatively looser and larger nucleus. As far as the available facts are valid it seems that plasmacytoid reticulum cells are present and are formed in the red pulp. The red pulp of the spleen thus plays a part in infective diseases by producing certain anti bodies and some globulins of the γ group whereas numerous lymphatic plasma cells are formed in the follicles (white pulp) whence they pass into the blood stream.

Ectopic Foci of Blood formation in the Spleen

It has long been held (Naegeli) that in adult life the spleen has lost its embryonic power of producing erythroblasts and myelocytes. Spleen puncture has shown that erythroblasts are rarely found in the normal spleen of adults but that a few erythroblasts and myelocytes are present in all infective splenomegalies and sometimes in larger numbers in hemolytic anemias. It seems clear that the spleen can regain erythropoietic powers although this can be of no great significance in health because the number of erythroblasts is small. On the other hand distinct foci of formation of red cells and granular leucocytes develop in diseases in which the medullary spaces of the bones are decreased in size, as in osteosclerosis and some tumour like states when there is usually a considerable degree of splenomegaly.

The existence of scattered granulocytes and even of myeloid foci in adult spleens has been known to pathologists for many years. Sternberg (1906) mentions them as being a variable but normal constituent of the spleen pulp and these observations were confirmed in the spleens of cases of sudden death and of executed criminals (Weidenreich 1911, Weill P 1920 and Bertelsen 1938). Hartmann also agrees that a few immature granulocytes are normally present in adults and careful examination of films from our own series of spleen punctures has revealed a few such elements in almost every case. The number is small in slight infections but greater in septic conditions.

These observations together with the evidence of tissue culture and the knowledge that marrow tissue may be found in areas of secondary calcification (e.g. in the lungs *lit see Lang*) show that in certain conditions of post natal life the undifferentiated mesenchymal cells can again give rise to the manifold types of blood cells. This subject is further discussed in the sections on splenomegalies.

4 CELLULAR STRUCTURE OF SPLEEN FLUID

The cells seen in films from spleen puncture are partly blood cells and partly derived from the splenic tissue itself. As a rule the majority are lymphatic elements with some cells from the reticulo endothelium and perhaps some from hemopoietic foci. Infections alter the composition so considerably that the films may show an

PLATE I A NORMAL RETICULO-ENDOTHELIAL CELLS IN SPLEEN PUNCTURE
(from a water colour picture by Mrs Bollinger Schudel)

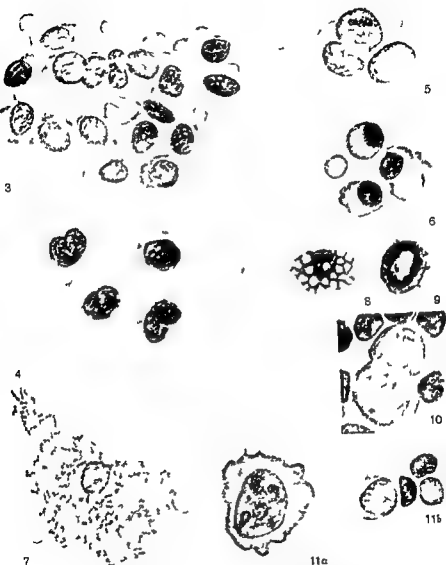


FIG 3 Serosa cell syncytium
FIG 4 Pulp cell syncytium
FIG 5 Separate serosa cells
FIG 6 Plasmacytoid reticulum cells
FIG 7 Large blue pigment macrophage
FIG 8 Lipophage

FIG 9 Tissue mast cell
FIG 10 Large lymphatic reticulum cells (germ centre cells)
FIG 11a Lymphatic plasmoblast
FIG 11b Lymphoblast and three old lymphocytes

various reticulum cells of the marrow and his four groups are generally accepted. He distinguishes macrophages, lymphoid reticulum cells, plasmacytoid reticulum cells and true endothelial cells.

Our own observations on gland puncture (Moeschlin 4) have led us to the following classification — (1) large lymphatic reticulum cells (2) foreign body and pigment macrophages (3) fat and lipid storage cells (lipophages) (4) lymphatic plasmoblasts (5) tissue mast cells. This classification has been adapted to fit the lymphatic reticulo endothelial elements in the spleen by adding the cells peculiar to that organ viz., serosa and pulp cells as well as those common to it and the marrow—capillary endothelium and plasmacytoid reticulum cells. We have attempted as has Rohr a grouping on purely morphological grounds ignoring functional subdivision into histiocytes, fibroblasts and fibrocytes. Rohr distinguishes the large lymphoid reticulum cells with immature loose nuclei from the small lymphoid reticulum cells with small dense nuclei. It seems likely that the larger forms with their immature loosely constructed nuclei correspond at least in part to the fibroblasts while the smaller ones are comparable with the older elements—fibrocytes and resting cells of the marrow reticulum.

The lymphoid reticulum cells possess the ability to develop into erythroblasts or even abnormal cells such as megaloblasts and epithelioid cells. Certainly *in vivo* studies of marrow (Nordensson, Rohr, Tischendorf *et al*) and gland puncture (Tischendorf, Stahel) confirm the occurrence of these changes.

We subdivide the reticulo endothelial cells found in spleen punctures as follows —

- (1) Serosa cells
- (2) Pulp cells
- (3) Small lymphoid reticulum cells
- (4) Macrophages
 - (a) cell debris macrophages
 - (b) pigment
 - (c) bacteria
 - (d) lipophages (fat and lipid macrophages)
- (5) Tissue mast cells
- (6) Plasmacytoid reticulum cells

1 SEROSA CELLS

Like other intra abdominal organs the spleen is covered by a single peritoneal layer of endothelium the so called serous coat. As spleen puncture involves only a small area of the serosa very few of the cells are found in films, but unlike other elements they are usually seen in small groups. If ascites is present a small amount of the fluid may enter the needle and then a few isolated serosal cells sometimes showing degenerative changes may be seen as in films of ordinary ascitic fluid.

Structure The cells are large ($20-40\mu$ in diameter) and are usually found in groups (Figs 3 5 12 a). Their characteristic structure distinguishes them from all other cells although there may be a slight resemblance to those of the pulp which however have greyer cytoplasm.

The cell is large and round without a distinct cell membrane and contains a round or oval nucleus which is often eccentric. Its size varies from $12-24\mu$ and its basichromatin is rather coarse and close sometimes

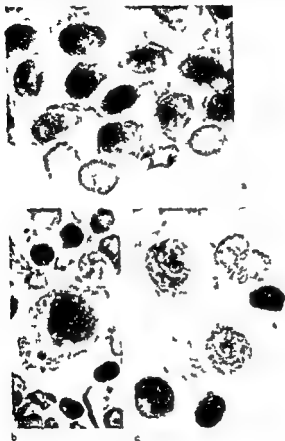


FIG. 12

(a) Serosal syncytium from spleen puncture. (b) c) Pulp cells characteristic of spleen puncture. (b) Shows a single cell containing ingested pigment. (c) A group of incompletely separated cells.

with 1 or 2 nucleoli 3μ in size. These are seen as pale areas which stain bluish in the younger forms. The cytoplasm stains pale blue and often contains evenly scattered fine azurophile granules. We found these cells only 11 times in 170 spleen punctures (see photomicrographs in Fig. 12 a and coloured picture 3).

2. PULP CELLS

These cells demand attention because they have not previously been described during life. Lubarsch mentioned certain elements

them from the tissue fragments. They are most numerous towards the end of the film often in a sort of reticular distribution of small but dense collections. That such elements are not scanty in the tissue itself is clear from histological sections which are seen to contain many small almost naked nuclei very like erythroblasts and small lymphocytes. For this reason these cells cannot be included in the qualitative splenogram.

The cells are small with almost naked round or slightly oval nuclei. The chromatin is almost homogeneously arranged in a solid mass in which nucleoli can be detected in the most immature forms. As said

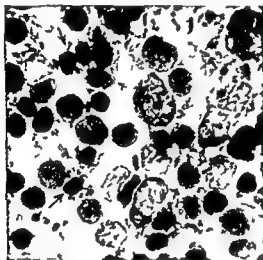


FIG. 13. Small type of reticulum cells scattered among normal lymphocytes. Frequently it is not possible to distinguish these elements from old lymphocytes.

above the cell can often not be distinguished from old small lymphocytes seen in spleen smears.

Similar cells are found in gland punctures. They are probably slightly differentiated mesenchymal cells which can develop into macrophages or fibroblasts. It is also possible that such stimuli as for instance inflammation may cause their conversion into lymphoblasts, erythroblasts and perhaps myeloblasts, thus explaining the genesis of ectopic foci of hemopoiesis.

In spleen films we have not been able to recognise the large lymphoid reticulum cells that Rohr described in marrow films, but this must not be confused with our large lymphatic reticulum cell as seen in spleen films (Figs 10-19). These latter are found in both spleen and gland films, probably corresponding with those from the germ centres, hence our inclusion of them in the lymphatic series.

found in post mortem smears which may be identical with the cells here described. Probably the elements we term pulp cells correspond with those known to histologists as spleen sinus cells or as sinus endothelium. These cells are found in all films from the spleen and their presence in films is proof that the material is derived from that organ. Neither in the marrow nor in lymphatic glands are identical cells found and we consider them to be the only morphological element peculiar to the spleen although their specificity has not previously been recognised because they were often supposed to be monocytes e.g. by Weil. Once their exact structure has been recognised the differences between them and other cells presents no difficulty.

In normal spleen films they form 0.2-0.6 per cent of the cells but rise as high as 7 per cent in inflammatory conditions.

They are often seen as indistinctly bounded elements with irregular edges to the cytoplasm probably because in health they form a firm cellular tissue becoming free only in abnormal conditions e.g. infections. Occasionally they are found in connected groups of 3 or 4 cells (Fig. 12 c) but the majority lie isolated among other types of cells.

Structure The cell body is usually elongated reaching a length of 40-50 μ , sometimes with a tail like projection at the ends. The nucleus (15-20 μ) usually lies eccentrically and is sometimes slightly indented or even almost lobulated thus somewhat resembling younger monocytes. The finely granular but closely packed chromatin which stains bluish violet only possesses nucleoli while it is still in the immature basophilic stage.

The cytoplasm is grey unlike that of the serosal cells sometimes with patches of reddish violet and usually contains fine granules varying from reddish violet to grey (see Fig. 4 on Plate I and b in Fig. 12). In a few darker and coarser granules are seen probably representing ingested ferruginous pigment. Our view is that these elements probably develop into typical macrophages certainly an uninterrupted series of transitional forms can be recognised.

3 LYMPHOID RETICULUM CELLS

As already mentioned the framework of the marrow glands and spleen is made up of histologically undifferentiated reticulum (mesenchymal) cells which are also known as fibroblasts and fibrocytes. Rohr distinguishes two types large and small. The latter (small lymphoid reticulum cells) are undoubtedly present in spleen films although they cannot always be distinguished from old or atrophied lymphocytes. It is only when the large lymphocytic type predominates (Fig. 13) or these cells lie among the myelocytes in cases of myeloid leukemia that they are recognisable with certainty.

As in the case of marrow films the cells that form the supporting tissue are scanty or crushed because of the difficulty of separating

occur in glands liver marrow and spleen. Hemosiderin macrophages in the marrow have been described by many writers

The *hemosiderin storage cell* (Fig 14 e) varies in size from 20 to 50 μ . The nucleus is small and usually oval lying eccentrically. The whole of

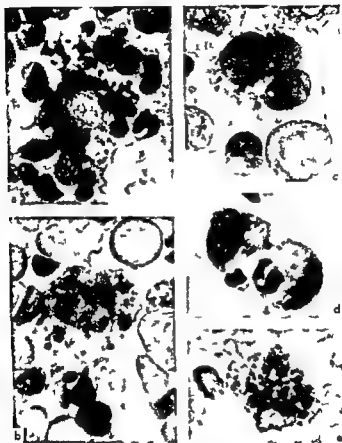


FIG 14 Cell-debris and pigment macrophages

- (a and b) Large macrophages loaded with nuclear remnants and pigment
 (c) Macrophage with 3 nuclei from a case of splenomegalic cirrhosis
 (d) Macrophage with ingested leucocytes. The pyknosis in the latter indicates that there has been phagocytosis and that the appearance is not an artefact
 (e) Hemosiderin macrophage from a case of chronic hemolysis

the cytoplasm is closely packed with rather coarse often slightly angular masses of dark brown or yellowish green pigment which do not usually invade the nucleus. No sharp cell outline can be found in most cases because the cells have been torn out of their reticular connections. For the same reason portions of protoplasm filled with masses of pigment but containing no nucleus are common. The cytoplasm is almost colourless and often invisible.

4 MACROPHAGES

Probably all reticulum and possibly endothelial cells are facultative macrophages. Certainly, in spleen films every stage intermediate between small reticulum cells and pulp cells on the one hand and macrophages on the other can be recognised. It has been supposed that the plasmacytoid reticulum cells possessed no phagocytic powers but Dubois Ferriere has shown experimentally that particles of carbon are found in them after injection of Indian ink. And in sternal marrow Rohr has observed transitions from these cells to lipophages. In aplastic anemia with early hæmosiderosis due to repeated transfusions we have seen iron pigment in these elements.

The macrophages can be subdivided into four groups according to the type of ingested material although mixed types do occur —

- (a) Cell debris macrophages
- (b) Pigment
- (c) Bacteria
- (d) Fat and lipid (Lipophages)

In sections, macrophages are seen in both the red pulp and the lymphatic nodules of the spleen. Those that contain cell debris are identical with similar elements in the marrow and glands but, in the spleen, certain pigment and fat macrophages of a type not found in those organs may be seen.

(a) *Cell debris Macrophages* Such cells are also found in gland punctures (Forkner Tischendorf Stahel Moeschlin (4) Stuyt Strunge) and in marrow films (Rohr *et al*). They are a constant feature of spleen films (0.1–0.2 per cent) increasing to 0.8 per cent in infective conditions.

Every kind of transition from smaller forms (20μ) to giant ones of 60μ can be found. The ingested material is usually nuclear or cytoplasmic detritus but may consist of a whole cell (Fig. 14 a) while an occasional cell is seen to contain carbon or to be encrusted with calcium.

Only cells showing signs of degeneration (nuclear pyknosis cytoplasmic vacuolation diffuseness of outline etc.) are ingested and we believe that the published pictures of whole cells inside megakaryocytes represent artefacts.

The structure of the nuclei of these macrophages resembles that of the lymphoid reticulum cells but in the older forms is extremely dense. In the young types the rather loosely arranged nucleus is central and may contain a small blue nucleolus while in old forms it is more elongated and lies at the edge of the cell (Fig. 14 a b).

(b) *Pigment Macrophages* This group includes all those cells that have picked up break down products of hæmoglobin or other pigments. There are two main groups viz those with iron containing and those with iron free pigment both of which are well known to

Gosio found cells laden with very dark melanin in spleen puncture (see Fig 33 *b c*) This pigment is quite different from the brownish or greenish yellow tint of hemosiderin and its presence is a valuable sign of malaria even many years earlier

(*c*) *Bacteria Macrophages* Schilling and more recently Rohr noted the occurrence of endothelial cells and of monocyte like elements of reticular origin in the blood in infective states (endocarditis) Ingested bacteria may be seen in the cytoplasm for instance we have seen a reticulum cell containing morphologically recognisable pneumococci in a spleen puncture performed during severe pneumonia (Fig 15 *b*) We have also seen a chain of streptococci in a monocyte (Fig 15 *c*) The discovery of such bacterial phagocytosis supports the view that the spleen plays an important defensive part in transient and more chronic bacteremias thus being of importance in both humoral or cellular immunity

Parasite Macrophages For the parasites of malaria phagocytosed in reticulum cells (see Gosio) and of Kala Azar (see Fig 33 *d*) Refer to the chapters on these diseases in the second part

(*d*) *Fat and Lipoid Macrophages (Lipophages)* Fat and lipoid storage cells are scanty in the spleen (1-2 in every 10 000 cells) in one case of sepsis lenta we found eight such cells in a whole film We have previously mentioned their occurrence in gland punctures so that it seems probable that those in the spleen are derived from its lymphatic follicles i.e. from its reticulum cells Their presence in the spleen has long been known (Lubarsch Kusunoki) but they have not previously been recognised in films In sternal marrow Rohr showed that both small reticulum cells and plasmacytoid reticulum cells could gradually become large fat cells and in spleen films it is possible to find every kind of transitional form First the fat globules in the cytoplasm are small and scanty but ultimately these elements become converted into large fat cells (Fig 16 *a-d*)

The nucleus of the large cells lies near the edge of the cells as in the case in the marrow A few macrophages however stain faintly and contain closely packed small vacuoles in the cytoplasm probably indicative of lipoids

In the larger elements the nucleus is usually central (Fig 16 *e f*) These are probably identical with the cells we described in gland punctures (large lipophages)

Large Monocytoid Macrophages We found these elements (Fig 16 III *i* and *k*) most often in chronically inflamed spleens and Gosio has described and illustrated similar cells from the spleen in chronic malaria They store pigments such as hemosiderin and melanin as well as lipoids Their origin is probably from various reticulo endothelial elements

They are quite large cells with indistinct edges The nucleus is slightly indented and the chromatin although fine is densely arranged usually

Blue Pigment Macrophages In a case of splenomegaly in a man aged 29 of uncertain but not hemolytic nature we found macrophages which contained closely packed fine granules which stained deep azure blue (Fig 15 a and Plate I Fig 7) Such blue pigment macrophages have not previously been described in gland or marrow punctures but Dr Brausil, of Zagreb has sent us a marrow film in which identical cells together with large lipophages

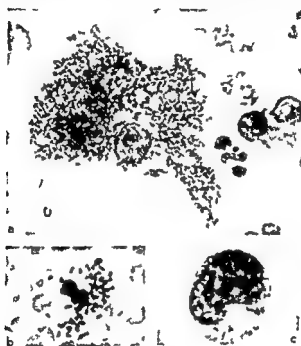


FIG 15

- (a) Large so-called blue pigment macrophage These cells are filled with the pigment that stains azure blue with May Grünwald (see Fig 7)
 (b) Bacteria macrophages
 (c) Monocyte with chain of cocci from endocarditis lenta

and many plasmacytoid reticulum cells were present The specimen was from a case of suspected chronic malaria

If such blue masses escape from crushed cells they may be mistaken for masses of bacteria or precipitates of stain Unlike the hemosiderin cells these elements usually have a round central nucleus which often contains a definite nucleolus

The blue stained granules are very fine and may be so closely packed that the whole cell body appears blue then the granules can only be recognised at the edges The nucleus is usually free from granules The cells vary from 30-60 μ

The nature of the pigment is unknown but we suspect that it is an iron free derivative of haemoglobin

Malaria Melanin Macrophages In cases of chronic malaria



FIG 16

III

(l) *Large monocyto-d macrophages* in a chronic inflammatory spleen. The monocyto-d nucleus with cytoplasm containing fat and other ingested materials is characteristic of these cells which somewhat resemble Gaucher cells ($\times 1,700$)

(k) *Histiocytic monocytes* from the spleen with denser arrangement of chromatin than in myeloid monocytes. These cells are particularly numerous in chronic inflammatory spleens ($\times 1,000$)

with single pale areas. The cytoplasm resembles that of Gaucher cells and stains pale reddish violet with small cloud like areas of pallor in contact with one another (Fig 16 III l and k)

5 TISSUE MAST CELLS

These cells are scanty (1 in about 10 000 cells) but are increased in chronic inflammations *e.g.* we saw 0.1 per cent in sepsis lenta and 0.2 per cent in lymphatic leukaemia

Rohr (1948) observed increase in the marrow in aplastic anaemia and also in a peculiar case of chronic interstitial inflammation of the marrow

They are not to be confused with the basophiles of the blood (Weil Strunge *et al*) which are essentially different from the mast cells of the spleen marrow and glands. There are a number of distinctive differences that indicate their genetic and functional disparity. The basophiles of the blood develop from basophile myelo-

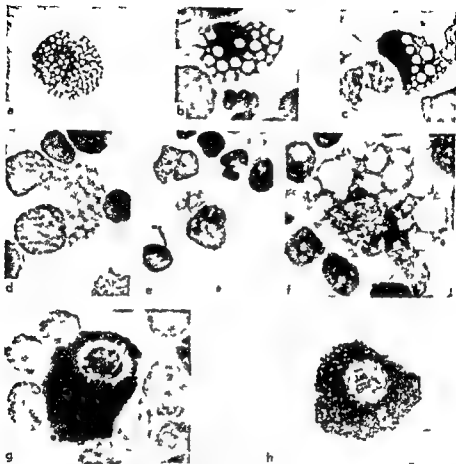


FIG 16

I Fat and lipid macrophages

- (a) Plasmacytoid reticulum cells showing early phagocytosis of fat
 (b, c, d) Typical fat cells
 (e and f) Large lipophages with central nuclei similar to those common in glands but scanty in marrow

II Tissue mast cells

- (g and h) Note the fine granules that do not involve the nucleus and compare with blood basophiles with their larger granules and narrower cytoplasm

The nucleus is usually eccentric and relative to the cytoplasm is small. It is rounded and in older specimens shows a cart wheel distribution. Only the younger cells show the presence of a small single nucleolus. Multi nucleate forms are not uncommon in chronic inflammation (Fig 17 *b* and *c*). Some contain fat globules and so represent transitions between the plasmacytoid reticulum cells and the large lipophages. They may also pick up hemosiderin (Fig 17 *d*) and as Dubois & Ferrière has shown particles of injected Indian ink.

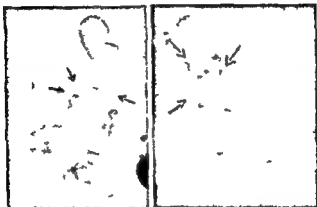


FIG 17 *d* Phagocytosis of hemosiderin in plasmacytoid reticulum cell

The distribution curve of the sizes of these cells is the same in spleen and marrow films (Comparative measurement each 50 cells in marrow and spleen). This fact together with the morphological evidence demonstrates their identity (Fig 18).

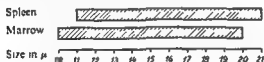


FIG 18 Variations in size of plasmacytoid reticulum cells

Structural Differences between the Plasmacytoid Reticulum Cell and the Lymphatic Tissue Cell

The reasons for distinguishing two types of plasma cell have already been discussed (see p 13) but their morphological differences deserve consideration.

The plasmacytoid reticulum cell (Fig 6) has a more violet blue cytoplasm and in the younger ones a narrower than in lymphatic plasma cells. The nuclei of the youngest stages of the former type are smaller and more compact with coarser chromatin than the delicate structure of the young lymphatic plasma cell (plasmoblast). The latter shows 3-5 distinct pale blue nucleoli which persist into

cytes *i.e.* they belong to the myeloblast series whereas the tissue mast cells which never appear in the blood, arise locally in various tissues arising directly from reticulum cells. Pappenheim Maximow, Naegeli *et al* showed that they do not give the oxydase or indo phenol blue reactions as do the hemic basophiles. Possibly the tissue mast cells play a part in regulating the coagulability of the blood by producing heparin as shown by Holmgren and Hilander (1937) and confirmed by Jorpes (1947) and Paff. Bloon and Reilly (1947). Ehrlich and co workers (1949) positively proved the production of heparin in these cells.

Tissue mast cells ($14-25\mu$) possess a redder finer and more closely packed granulation than do hemic basophiles. The cytoplasm is fairly wide and the granules lie round the nucleus (Figs 9 16 g h) whereas in the basophile leucocytes the coarser and darker granules are scattered over the lobulated nucleus.

6 PLASMACYTOID RETICULUM CELLS

The plasmacytoid reticulum cells seen in spleen films are identical with those found in the marrow. Their numbers vary from 0.1 to 0.3 per cent in normal conditions to 10 per cent or even 17 per



FIG. 17

(a) Plasmacytoid reticulum cells of the same type as those of the marrow.
(b and c) Two large multi-nucleated cells in which mitosis was not followed by division of the cytoplasm.

cent in chronic inflammatory conditions. They are also increased in hepatic cirrhosis.

In films and sections they are usually found in groups of 2-5 cells. For discussion of their functions and for the findings in the phase contrast see pp. 12-16 and Fig. 20 c d e.

Structure. In the spleen the size varies from 11 to 21μ . The cytoplasm is usually rather more violet blue than the azure blue cytoplasm of the larger lymphatic plasma cells and is often riddled with vacuoles but less often contains azure granules. The edge is indistinct and rough.

as seen in sections especially because of their characteristically vesicular nuclei their identity must be admitted

Some writers call these large vesicular cells of the germ-centres macrolymphoblasts* (Hartmann) while others regard them as being reticulo endothelial (see Bloom) We are convinced that study of gland and spleen punctures clearly show transitions from these elements to lymphoblasts and plasmoblasts i.e. they are the parents of the hemic plasma cells while they can also be converted into glandular fever cells (lymphoid monoblasts) The large lymphatic reticulum cells of the germ centres are thus to be regarded at least in part as the reticulo endothelial stem cells of the lymphocytic series

In normal circumstances lymphocytes regenerate from lymphoblasts but in conditions of stress the large lymphatic reticulum cells from the interior of the germ centres probably become active and give rise to lymphoblasts and plasmoblasts These lymphatic cells differ from the large lymphoid reticulum cells of the marrow in the presence of much looser chromatin in their large nuclei with very distinct large nucleoli They differ from lymphatic plasmoblasts by their central nuclei with finer masses of chromatin enclosed in a narrow zone of cytoplasm whereas they can be distinguished from lymphoblasts by their size although transitional forms are also found

2 LYMPHOBLASTS

Normally only 0.1-0.2 per cent are found in spleen films and it is surprising how few are found in most lymphatic leukæmias (see p. 122)

These cells are 11-18 μ in diameter The pale blue cytoplasm is rather narrow and the nucleus has a fine but close network of chromatin in which 1-2 distinct nucleoli are visible (Fig. 11 b) The main distinction from the larger myeloblasts lies in the greater density of their chromatin

3 LYMPHOCYTES

(a) *Young Small Lymphocytes* These are fairly small cells with narrow cytoplasm and compact nuclei in which 1 or 2 pale areas or even nucleoli can be seen Normally they form 0.6-7.5 per cent of the total cells

(b) *Mature Small Lymphocytes* These elements have dense nuclei with no trace of nucleoli

(c) *Young Large Lymphocytes* In this group (0.4-3 per cent) are included all the larger cells with wide cytoplasm (13-18 μ) and either a nucleolus or a circumscribed pale area in the nuclei

(d) *Mature Large Lymphocytes* These are the elements (2-5 per cent) with rather wide cytoplasm (11-17 μ) and no distinct pale area in the nuclei

a fairly mature stage while even the least mature examples of the plasmacytoid reticulum cell have only one small nucleolus

Even when mature the lymphatic plasma cell retains the pachy chromatic structure of the nucleus which lies more eccentrically than that of the plasmacytoid reticulum cell. Even the mitoses differ (see ¶ 38 and Fig 23 *a b c d*)

Phagocytosis of lipoids and hemosiderin (Fig 17, *d*) may be present in the plasmacytoid reticulum cells but is unknown in lymphatic plasma cells

II LYMPHATIC SERIES

1 LARGE LYMPHATIC RETICULUM CELLS

In punctures of inflamed glands we found a few elements that we call large lymphatic reticulum cells (Figs 11 *a* 19). These are large (20–25 μ) with narrow dark blue cytoplasm and indistinct outline, while the large round or oval nucleus contains 3–5 nucleoli in its delicate chromatin. As far as can be ascertained from pictures published by others these are identical with the hæmocytoblasts found in gland puncture by Pavlowsky, the undifferentiated endothelial reticulum cells of Tischendorf and the young round cells of Stahel.

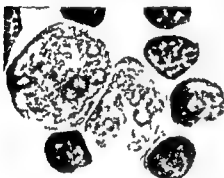


FIG 19 Large lymphatic reticulum cells (germ-centre cells) with a narrow dark blue cytoplasm and a large nucleus. These cells can be found in spleen films and in gland punctures and are identical with the germ-centre cells.

A few of these can be found in spleen punctures and where a follicle has been penetrated and spread out it is clear that these cells belong to the lymphatic tissue of the spleen. They form 0.01 per cent of the cells of the normal spleen but may rise as high as 1 per cent in lymphatic reactions.

Their structure indicates that they are only slightly differentiated young cells which probably arise from the reticulo endothelium of lymphadenoid tissue. They are not found in marrow films. Our studies on gland puncture and spleen puncture seem to us to show that these elements are the constituents of the germ centres of the lymphatic follicle. They are identical with the germ centre cells described by Thiel and Downey in histological sections. Certainly their number is increased whenever germ centres are numerous. As no other cell found in films could be identical with these elements

Structure The plasmoblast is a fairly large cell (17-30 μ) of rounded or slightly oval shape with a central or eccentric large round nucleus (12-25 μ). The cytoplasm stains cornflower blue with May Grunwald often with a pale area on one side of the nucleus especially in the more mature forms (Figs 11 a 20 a).

In the young forms the cytoplasm is bulky and the cell membrane



FIG. 21. Development of lymphatic plasma cells based on gland puncture in German mealies. (See *Helv. et Med. Acta* 7: 377). An identical series can be detected in spleen films.

so delicate that it is easily injured and hence apparently separated pieces of cytoplasm or cells with torn edges are often seen. Some have vacuoles in the cytoplasm similar to those seen in mature hemoc plasma cells.

We have never found any granules whether with May Grunwald Janus green pyrodim or the peroxidase method or the phase contrast microscope but the cytoplasm of some of the deeply basophilic cells is distinctly cloudy.

Such subdivision of the lymphocytes is convenient in differential counting but transitional forms are quite common

4 PLASMOBLASTS

The origin and nature of these cells has already been discussed (pp 12-16)

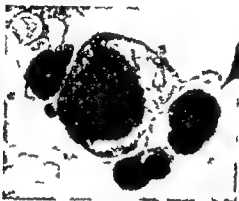


FIG 20 a Large young lymphatic plasma cells (*Plasmoblast*) with typical eccentric nucleus and large cornflower blue cytoplasm in spleen film of epidemic hepatitis

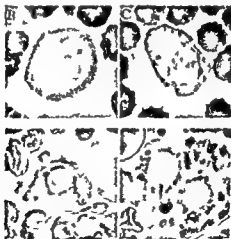


FIG 20 b c d e Phase contrast pictures of plasma cells The plasmacytoid reticulum cells show a dark drop like granulation which is much coarser but more plentiful in a case of myeloma living in contact with the nuclear membrane Lymphatic plasma cells do not show these structures It is possible that the granules represent certain protein substances formed by the reticulum cells (Zeiss phase contrast microscope 1000)

(b) Lymphatic plasmoblast in the blood showing nucleoli (from a case of Rubella)

(c) Mature lymphatic plasma cell in the blood (from a case of Rubella)

(d) Plasmacytoid reticulum cells in the bone marrow

(e) M-clema cells

strikingly large showing compared with the condition of the marrow a definite delay in maturation

We have only found myeloblasts in cases of leukemia

It was previously assumed that there is a continuous development of all series from the myeloblasts and premyelocytes to the mature granulocytes that appear in the blood. Rohr has asserted that the staff forms are mainly produced from metamyelocytes which in turn arise from continued division of myelocytes. Thorell (1944) believes that the myelocytes are mainly produced by continued new formation and maturation of myeloblasts and premyelocytes because he was able to detect a large increased formation of plasma proteins only in these cells. The complete absence of myeloblasts in the spleen seems strongly to support Rohr's view that the granulocytes are normally formed by continual division of the myelocytes.

Megakaryocytes We found them in only 10 out of 220 spleen punctures (excluding the leukemias) including 1 case of hemolytic anemia associated with undulant fever (up to 10 cells per slide) and also in sepsis lenta inflammatory cirrhosis 2 cases of thrombocytopenia (Fig. 28) and in osteosclerotic anemias. In chronic myeloid leukemia megakaryocytes may be scanty or occasionally numerous.

D MATURE GRANULOCYTES

The distribution of the mature granulocytes in normal spleen is shown in the section on the splenogram. It is however necessary to call attention to a few cells of the monocytic group with more compact nuclei and coarser granules than those found in the blood (Fig. 16 A). These probably correspond with the reticular monocytes (Rohr) i.e. cells which have arisen from the reticulo endothelium perhaps through the pulp cells. In cases of lymphatic reaction lymphatic monocytes are fairly frequently found. These have a compact and bluish broad cytoplasm usually with few or no granules and with a general resemblance to glandular fever cells. As these myeloid lymphatic and reticular monocytes in spleen punctures cannot always be distinguished from one another and as they are always rather scanty we have included them in the total number of monocytes only separating lymphatic forms in cases of glandular fever cells i.e. definitely pathological forms.

E THROMBOCYTES

As Weil has previously pointed out platelets are more numerous in spleen puncture than in the blood. We have tried to enumerate these elements but found that it was impossible because of very rapid coagulation. The spleen seems to play a significant part in the regulation of the platelets partly as in the case of blood cells in general

The nuclei of the young types are very characteristic. The chromatin is in fine granules arranged in a delicate network in which 2 to 5 pale nucleoli are clearly visible. There is a slight resemblance to the nuclear structure of young megaloblasts. The nucleoli are striking because of their size and distinctness. Usually there are 3 or 4 large ones, but occasionally there are up to 7 smaller ones. With May Grunwald staining the nucleoli are pale but sharply defined (Fig 11 a).

Mature Lymphatic Plasma Cells Both in gland and spleen punctures it is possible to find every stage of transition between plasmoblasts and mature lymphatic plasma cells. The latter differ from the plasmacytoid reticulum cells especially by their larger nucleus and finer chromatin structure (Fig 21). As a rule the younger types, *i.e.* with remnants of nucleoli, are more numerous (Fig 21 a b c).

C IMMATURE MYELOID CELLS FROM HÆMOPOIETIC FOCI IN THE SPLEEN

There is no necessity to describe the erythroblasts, myelocytes and occasional megakaryocytes that are found in punctures of spleens in which hæmopoietic foci are present.

The erythroblasts in inflamed spleens varied between 0.2-15.15 per cent and consisted mainly of polychromatic and ortho-

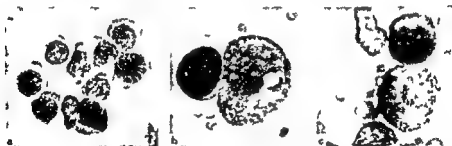


FIG. 22. Foci of ectopic myeloid hæmopoiesis.

(a) Group of erythroblasts from the spleen in chronic inflammation.
(b and c) Partially mature myelocytes: the former from a case of lymphatic leukaemia; the latter from a hæmolytic anaemia.

chromatic types (Fig 22 a) although many basophile ones could be found. With care it is possible to find every transitional stage between the typical basophilic erythroblasts and the mature orthochromatic elements. Occasionally even basophile macroblasts are seen (Fig 27 a).

Myelocytes (Fig 22 b c) are on the whole less numerous than erythroblasts in the normal spleen (0.1-0.2 per cent). In cases of inflammation they may rise to 2.7 per cent, and in osteosclerosis as high as 25 per cent. In the former condition the myelocytes are

G PATHOLOGICAL CELLS IN SPLEEN PUNCTURE

Descriptions of these elements will be found in the discussions of the various diseases. Here we only give a list of the pathological cells that may be present in spleen puncture and from which diagnostic inferences can be drawn

(1) *Pathological inflammatory cells of the reticulo endothelium*

Glandular fever cells

Epithelioid cells

Langhans giant cells } (Tuberculosis Morbus Boeck)

Bang's epithelioid cells (undulant fever)

(2) *Neoplastic cells*

Sternberg Reed cells (Hodgkin's disease)—probably neoplastic

Lymphosarcoma cells

Sarcoma cells e.g. reticulo sarcoma

Myeloma cells

Leukæmic cells—viz. abnormal types e.g. para myeloblasts etc. in acute and chronic leukæmias

Carcinoma cells—extremely rare in the spleen but may be found if e.g. a hypernephroma or pancreatic tumour is punctured by mistake

(3) *Pathological lipid storage cells*: Gaucher and Niemann Pick diseases

H MITOSES IN SPLEEN PUNCTURES

Since Fieschi's admirable observations on cell division in the sternal marrow hæmatologists have devoted much attention to the peculiarities of mitoses and the influence of internal and external factors on them. A study of mitoses in spleen puncture is also valuable particularly in the leukæmias and we would therefore first discuss the structure of the individual forms. It must be admitted however that the allocation of the individual mitoses to definite cell groups requires considerable practice especially in the case of non granular forms. It is specially to be noted that the cytoplasm of lymphoblasts basophile erythroblasts and cells of the reticulo endothelium is strikingly granular during the monaster and diaster stages (Fig 23 c d i) and it is possible therefore to confuse them with myelocytes although there are differences in the chromosomes

Mitoses in Reticulo endothelial Cells Serosa cells. These elements are scanty and it is a great rarity to detect mitoses in them. We were able to find a definite prophase in only 1 case (Fig 23 a)

Pulp Cells and Tissue Mast Cells We have never detected unquestionable mitoses in these elements

by hormonal inhibition of the marrow (Naegeli *et al*) and partly by acting as a reservoir (Heilmeyer). Probably the spleen also removes and destroys effete platelets. Certainly in some forms of thrombocytopenia both inhibition of the maturation of megakaryocytes and increased destruction of platelets occur.

F CELLS OF LUNG ALVEOLI

It has been mentioned that a small amount of pulmonary tissue may be aspirated if the puncture is performed at too high a level or if the costo pleural groove extends abnormally low. This has happened three times in our experience and as the cells found in films may arouse suspicion of neoplasia it is important to recognise their features.

Structure. In films pulmonary alveolar cells usually form larger or smaller groups. The individual cells vary from 18 to 34 μ while the boundaries are indistinct or even absent so that some of the cells seem to have 2 or 3 nuclei. The nucleus (11 to 20 μ) is round and usually eccentrically placed. It contains 1, 2 or rarely 3 distinct nucleoli which are well demarcated from the nuclear chromatin by a dark ring. The latter is fairly dense but areas of different density can be detected so that there is some resemblance to the nucleus of the mature lymphocyte. The nuclear membrane is usually visible as a dark line. Occasionally a small accessory nucleus is present. The cytoplasm varies from reddish violet to pink and may contain a few granules of carbon or hemosiderin (Fig 22 d).

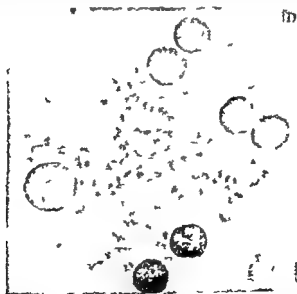


FIG 22 d. Cells from lung alveoli. The arrangement in smaller or larger groups and the indistinct boundaries are characteristic. The nucleus is round and with small sharply demarcated nucleolus. The cytoplasm is blue and in the present example contains hemosiderin (heart failure cells).

chromosomes of lymphatic plasmoblasts (Fig 23 *b*) Plasmacytoid reticulum cells and lymphatic plasma cells are thus distinct in the structure of their mitoses

Large Lymphatic Reticulum Cells : During mitosis the chromosomes are long and narrow with an acute angle (Fig 23 *e f*) They can only be distinguished from the mitoses of lymphoblasts by the size of the cells and the narrower cytoplasm No quantitative estimation of their frequency can be given because of the scantiness of these elements

Lymphoblasts The mitoses show rather delicate but short chromosomes (Fig 23 *g h i*) A typical lymphoblastic prophase is seen in Fig 23 *g* (a case of chronic lymphatic leukaemia) In comparison with the mitoses of other cell types the cytoplasm is narrow during the monaster and diaster stages

Plasmoblasts These cells with their azure blue cytoplasm and delicate dark violet elongated pairs of chromosomes in a distinct cytoplasmic rim show beautiful mitotic figures (Fig 23 *b*) and are only excelled by the delicate mitoses of megakaryoblasts As compared with the mitoses of myeloblasts and myelocytes the chromosomes show a fairly acute angle (Fig 23 *b*) Mitoses in such plasmoblasts are increased in infective hepatitis (6 mitoses per 100 plasmoblasts) and in chronic inflammatory spleens in sepsis lenta In gland punctures in German measles we found 1 to 3 plasmoblast mitoses per 100 lymphatic plasma cell and 1 to 6 per 100 plasmoblasts

Erythroblasts As erythroblasts are scanty in all spleen punctures except those from cases of leukaemia only few mitoses can be found In chronic myeloid leukaemia and particularly in so-called erythroleukaemia such mitoses are common Fieschi found 12-20 *pro mille* of erythroblast mitoses in the marrow while Rohr found figures as high as 42 *pro mille* and enumeration of the mitoses in a spleen puncture from a case of thrombocytopenia with 7.3 per cent of erythroblasts showed the following distribution —

	No of cells	Mitoses
Basophile erythroblasts	29	0
Polychromatic erythroblasts	30	2
Oxyphile erythroblasts	41	1
Total	100	3

The mitotic index of such extramedullary erythroblasts scarcely differs from that of the same cells in the marrow

Fieschi and Kienle have given detailed descriptions of the structure of erythroblast mitoses The chromosomes are broader and shorter

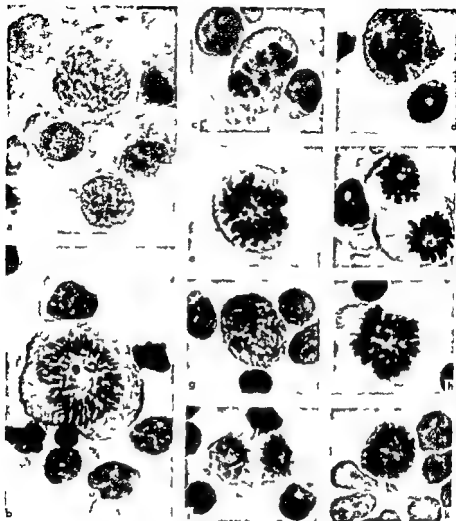


FIG. 23 Mitoses in reticulo-endothelial and lymphatic cells in spleen films

(a) Prophase in a serosa cell

(b) Metaphase in a lymphatic plasmoblast

(c and d) Mitoses in plasmacytoid reticulum cell

(e and f) Mitoses in large lymphatic reticulum cells

(g, h, i) Mitoses in lymphoblasts

(k) Metaphase in young lymphocyte (mitoses in such lymphocytes are extremely rare)

Plasmacytoid Reticulum Cells. Rohr has seen unquestionable mitoses of these cells in sternal puncture and so has controverted the view of Klima that these elements divide by amitosis. We have also seen occasional mitotic divisions in plasmacytoid reticulum cells in the spleen (Fig. 23 c d)

The chromosomes are relatively short and wide, occasional ones appearing adherent to one another. These mitoses with their small chromosomes are quite distinct from the large but relatively delicate

mitoses of lymphoblasts are scarce in gland punctures but a few may be found by careful search but they are exceptional in pro lymphocytes and are almost unknown in mature lymphocytes. Indeed we have only seen one metaphase (Fig 23 k) and in another case one dispireme in a young small lymphocyte. This is the more remarkable because as Rohr has shown in the granulocytic series typical mitoses occur in myeloblasts and even in staff forms but most plentifully in the more mature of the myelocytes.

We used films from a spleen surgically removed from a case of Felty's syndrome in which there was lymphocytic hyperplasia for comparison with films from 3 cases of lymphatic leukemia. With the assistance of Miss Ernst we obtained the following figures which are of course not to be regarded as absolute values. Even in spite of the margin of error the figures emphasise the great difference in the incidence of mitoses in the lymphatic and myeloid series.

Total cells examined	50 000
Lymphoblasts	12
Mitoses	2 (1 prophase 1 diaster)
Mitotic index	0.04 /
(= total of mitoses per 1 000 cells or 100 mitoses per 2½ million lymphocytes)	

In the marrow Rohr found 100 mitoses in 40 000 myeloid cells and 100 mitoses in 2 400 erythroblasts. *The number of mitoses in relation to the total number of lymphocytes is thus about 50 times less than in the cells of the myeloid series and 1 000 times less than in the erythropoietic series.* Even so in health about one sixth and in lymphatic leukemia about one fifth of the lymphoblasts appear to be dividing at any one time but these elements only form 0.2–1 per cent of the lymphocytes in the normal adenogram and about 0.6 per cent in lymphatic leukemia.

Explanation presents difficulties because there is no reason to assume that the number of lymphocytes lost and destroyed is less than that of granulocytes. Certainly the presence of so many lymphocytes in chronic inflammatory foci and exudates as well as in the gastro-intestinal mucosa suggests that the daily requirements of lymphocytes is high.

Fieschi has suggested that amitotic division of lymphocytes may occur but we have never seen anything to suggest it. In our opinion we do not believe that any proof exists for an amitotic division even in other hæmatopoietic cells.

We have a rough knowledge of the life span of mature granulocytes (2–3 days according to Osgood) and of mature erythrocytes

than those of myeloid cells. During prophase the cytoplasm is narrow (Fig 24 a) or even invisible but during the monaster and diaster stages it is obvious because of the shortness of the chromosomes (Fig 24 b) although less bulky than in myelocytes. It is not as easy to distinguish the individual chromosomes as it is in plasmoblasts megaloblasts and even in myelocytes because they are rather stuck together. Monasters in polychromatic erythroblasts are the most numerous of the mitoses.

Myeloblasts and Myelocytes An occasional mitosis is seen in the myelocytes in extra medullary foci and is easily recognisable because granules are present at all stages. The chromosomes are larger than those of myeloblasts and erythroblasts while they are thicker than those of



FIG 24 Erythroblast mitoses in spleen puncture from a case of myeloid leukemia

(a) Prophase (b) Monaster (c) Diaster (d) Dispireme

lymphoblasts and plasmoblasts. The width of the cytoplasm is considerable in all phases of mitoses.

Myeloblast mitoses which are only found in the spleen in leukemias differ from those of erythroblasts in the more delicate and more distinct chromosomes although these are less delicate than those of lymphoblasts. The cytoplasm is even narrower than in lymphoblasts during division.

Spleen films are specially suitable for a study of mitoses and it may be that a comparison with those in the marrow may throw light on the development and progress of the leukemias. The effects of such therapeutic substances as arsenic urethane and X rays on mitoses can be studied by serial spleen punctures (see Leukemia).

I THE LYMPHOCYTES THEIR MITOSES METABOLISM LIFE SPAN AND FUNCTIONS

Mitoses in the lymphocytic series are much less frequent than in the granulocytic and erythropoietic series. Even in lymphatic leukemia in which there is so great an increase of lymphocytes the scarcity of mitoses is remarkable. Nordenson Klima and Fieschi mention the rarity of mitoses in the marrow in lymphatic leukemia although they do not give any figures. Fieschi goes so far as to suggest that amitotic division must occur. We have noticed that

considerable life span for the lymphocytes. It is conceivable that lymphocytes may return to the lymphatic tissue to regenerate their cytoplasm after having parted with various protein substances. Dougherty (1946) has reached a similar conclusion. Dougherty and White have suggested that lymphocytes may play a part in the production of γ globulin. This is in contradiction to the work of Bing, Gormsen and Fagraeus which we have already discussed (pp 14-15) which seems to have shown conclusively that this is a function of the plasma cells and not of the lymphocytes especially in so far as anti bodies are concerned.

Lymphocytes probably play a significant part in digestive processes as their number in the intestinal mucosa and the chyle suggests.

The adrenal cortex seems to regulate both the formation and destruction of lymphocytes (Dougherty and White) a function which is of considerable significance in infections. Although production of anti bodies from the lymphocytes is in Gormsen's opinion unlikely there still remains the possibility that the lymphocytes are able to produce some other globulins.

A AMITOTIC CELL DIVISION

It is remarkable how uncritically and frequently amitosis has been invoked to explain the presence of two or more nuclei in a cell (Fig 17 *b* and *c*). It is true to say that we have no proof that amitosis is ever the explanation of such appearances.

Confusion has doubtless been caused by the fact that even in the final phases of mitosis as seen in films of the marrow, spleen and glands just before the chromosomes reunite into nuclei there may still be no signs of division of the cytoplasm. This is particularly common in pathological conditions in which abnormalities of mitosis occur e.g. haploid misplaced and pyknotic chromosomes or multipolar divisions (Fieschi, Rohr) in pernicious anaemia. In such circumstances the nuclei may still show signs of recent division and their absence cannot therefore be regarded as evidence of amitosis. All these cells with two or more nuclei are distinctly larger than the normal cells and this proves that division of the cytoplasm has not taken place.

The only proof of the possibility of amitosis in hemopoietic cells would be direct observation or a cinematographic record of its occurrence in tissue cultures. Certainly Kienle's contention that the absence of chromosome structure in multi nucleated cells is strong evidence of their amitotic origin cannot be accepted especially as he admits that such cells are usually found in conditions in which atypical mitoses are also found (see Kienle in Figs 29 and 30). Undritz shares our view that twin and multiple nuclei are not attributable to amitotic division (polyploid cells).

(4-6 weeks according to Heilmeyer 100-120 days according to Moeschlin (8) and Rohr and others) but the life span of the lymphocytes is purely conjectural. *But the infrequency of lymphocytic mitoses suggests a long life for these cells.* Of course mitoses in the relatively simple lymphocytes may proceed more rapidly than in the complex granulocytes. Moellendorff by a cinematographic method found that in tissue cultures fibroblasts divided in 50-70 minutes. Unfortunately there are no similar investigations on lymphocytes but perhaps this problem can be solved by observations on cultures of lymphocytes.

The mitoses in the spleen in lymphatic leukaemia are as follows —

	A. avg V I	Ca 1	Ca 2	Case 3 a d b
Total lymphocytes counted	3 354	2 971	3 09	4 127
Of these lymphoblasts		20	20	3 998
In mitosis	4	—	—	20
Prophase	1	—	—	3
Metaphase	3	—	—	2
Anaphase	—	—	—	—
Dispersing	—	—	—	—
Lymphoblasts per 1 000	6.08	6.73	6.60	4.92
Lymphoblast mitoses per 1 000	0.65	1.34	0	0.61
Lymphocytes per 100 lymphoblasts	16.770	14.855	15.145	70.312
Lymphocytes per 100 lymphoblast mitoses	121.504 (Cases I and III)	74.275	?	168.733

The comparison of these figures with normal values shows that the mitoses are 15-30 times more frequent in Cases 1 and 3 in which lymphoblasts were more numerous. Even so the relative number of lymphoblasts in mitosis viz. about one fifth of the cells is approximately normal. In Case 2 although the number of lymphoblasts was the same no mitoses were seen and it appears that such cases run a more prolonged course.

Function of the Lymphocytes. This is still obscure. Sjoval (1936) investigated rabbits which had been frequently bled and reached the conclusion that there is a perpetual interchange of lymphocytes between the lymphatic tissues and the blood i.e. that the lymphocytes can return to the lymphatic tissue. He did not find any increase of secondary lymph nodes and for this reason doubts whether the germ centres play any part in the production of lymphocytes. It appears to us that such repeated bleedings may have induced a deficiency of some substances required for the new formation of lymphocytes analogous to the deficiency of iron for the erythropoiesis that develops in these circumstances. His conclusions will therefore not stand criticism but they do indicate a

tissue penetrated is such as to ensure that most of the cells are derived from the sinuses. Both sections and films of aspirated material show clearly that both pulp and follicle cells are always present. Even if a small purely lymphatic follicle is spread in a film (Fig. 25) little error will result if the splenogram is made by counting at least 1 000 cells in several films.

The technique of spreading the material as well as that of enumeration can affect the splenogram. For instance some crushed cells are found in every film especially when abnormally delicate elements are present as in the leukemias. If therefore films are not made with as little pressure as possible the proportion of the older and less fragile lymphocytes will appear unduly high. The best parts of the films should be used for examination and some from the upper and lower parts as well as from the middle zone should be examined perhaps about 300 cells from each.

Tempka includes crushed cells in the splenogram but in our opinion it is better not to do so if the technique advocated above is used.

Table 1 shows splenograms from various cases in which counts were performed in different films from the same puncture —

TABLE 1

(Pp film)	Portal thrombosis		Sepsis lenta		Glandular fever		Chronic lymph leukemia		Chronic myeloid leukemia	
	Pp (a)	Pp (b)	Pp (b)	Pp (a)	Pp (a)	Pp (b)	Pp (a)	Pp (b)	Pp (b)	Pp (a)
Macrophages	—	—	0.3	0.7	+	—	—	—	—	—
Plasmacyt retic cells	0.3	0.1	3.0	1.0	1.1	1.4	—	—	—	—
Pulp cells	0.5	0.9	1.5	0.8	1.0	0.5	—	—	—	—
Erythroblasts basophilic	0.1	—	—	—	—	—	—	—	0.6	0.7
Erythroblasts polychr	—	—	±	—	0.1	—	—	—	3.1	3.5
Erythroblasts orthochr	—	—	—	—	—	—	0.1	—	2.9	1.9
Mycoblasts	—	—	—	—	—	—	—	—	2.4	0.6
Myelocytes immature	—	—	—	0.1	—	—	—	—	11.0	13.8
Myelocytes half mature	—	—	0.2	0.2	—	0.1	0.1	—	2.0	2.7
Myelocytes mature	0.2	—	0.6	0.2	—	—	0.1	0.1	3.6	1.4
Metamyelocytes	—	—	0.6	0.4	0.1	0.1	—	—	14.6	11.7
Neutrophils staff	4.0	5.6	7.9	9.6	7.4	8.8	0.3	0.3	27.4	26.0
Neutrophils segmented	12.9	14.6	6.2	7.9	4.0	4.4	1.2	2.1	10.4	6.8
Eosinophiles mature	2.3	1.4	—	0.1	0.2	0.7	—	—	1.1	1.7
Basophiles mature	0.2	0.3	0.1	0.2	0.2	0.2	0.1	0.1	4.0	2.6
Monocytes	2.7	2.8	0.7	1.9	4.6	4.2	0.2	0	0.5	0.8
Lymphoblasts	0.1	—	1.3	0.8	—	—	0.8	0.5	—	—
Lymphocytes small y ng	10.5	5.8	5.7	8.6	2.5	1.9	29.4	37.4	—	—
Lymphocytes small old	64.1	63.3	57.6	45.5	47.4	46.6	65.2	59.3	1.4	0.7
Lymphocytes large y ng	0.8	0.9	6.2	8.3	0.1	0.6	1.4	3.2	—	—
Lymphocytes large old	1.1	4.1	5.7	10.0	5.9	6.8	1	1.7	—	0.1
Lymphocytes total	76.6	74.1	76.5	73.2	78.6	78.1	98.0	97.1	1.4	0.8
Lymphoid monoblasts	—	—	—	—	4.5	1.1	—	—	—	—
Glandular fever mature cells	—	—	—	—	23.2	21.1	—	—	—	—
Plasmoblasts	+	—	0.3	0.5	0.1	—	—	—	—	—
Plasma cells half mature and mature	2	0.2	2.1	3.7	2.6	1.5	—	—	—	—

L THE SPLENOGRAM

When spleen puncture is performed purely for diagnosis it usually suffices to examine the films carefully without making a detailed differential count. In such conditions as aleukæmic lymphatic leukæmia with splenomegaly (splenomegalic lymphadenosis) it is advisable to obtain a full qualitative picture *i.e.* a splenogram. Then again in myeloid leukæmia a splenogram may be useful for comparison with the myelogram.

A considerable number of splenograms are recorded in Part II mainly for didactic purposes.

The earliest splenograms were published by Mele; later ones by

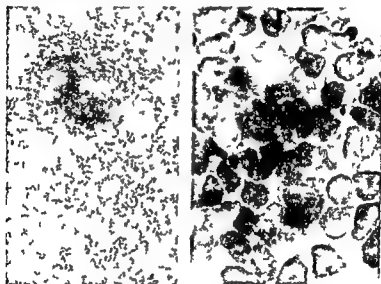


FIG. 25 Lymphatic follicle in spleen film low and high magnification

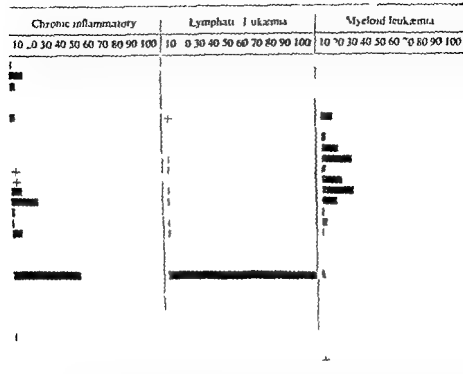
Weil and normal figures by Tempka. We have evaluated many splenograms and are convinced that at least 1 000 cells must be examined and classified while in order to diminish the margin of error as much as possible certain differential features must be kept in mind.

If our technique of puncture is properly followed the small amount of blood in the spleen juice is derived from the pulp sinuses. This does not cause confusion because the sinuses are not normally connected with the general circulation their cellular contents being typically splenic. It is only when there is a marked degree of portal hypertension that pure blood from the dilated sinuses enters the syringe. Even so in every case the splenogram should be judged in the light of the blood picture as a whole.

There is little likelihood of obtaining only material from a lymphatic follicle: the size of the needle and the depth of spleen

TABLE 4
Variations of Normal Splenogram

	Red	Temp
<i>Reticulum cells</i>		
Pigment and cell macrophages (1 per 10 000)	0 1-0 3 0-0 01	0 6-7 5 0 3
Fat cells	0 2-0 8	0 4 1 15
Plasmacytoid reticulum cells	0-0 1	
Tissue mast cells	0 2-0 6	
Pulp cells		
<i>Erythroblasts</i>		
Basophilic polychromatic orthochromatic	0-0 1-0 4	0
<i>Granulopoiesis</i>		
Myeloblasts	0	0
Myelocytes immature	0-0 1	
Myelocytes half mature	0-0 1	
Myelocytes mature	0 05-0 3	0
Metamyelocytes	0-0 1	
Neutrophiles staff	1 0-7 0	0 45 1 65
Neutrophiles segmented	8-25	5 8
Eosinophiles mature	0 2 1 5	0 12-0 6
Basophiles mature	0 1 1 1	0 17-0 5
Monocytes	1 7-2 4	0 5 0 5 8 6
<i>Lymphatic series</i>		
Large lymphatic reticulum cells	0-0 1	
Lymphoblasts	0-0 2	0 3-7 0 41-59 5
Lymphocytes		
Small young	0 6-7 5	
Large young	0 4-3 0	
Small mature	55-79 5	1 15-1 1 4 18 5-47 1
Large mature	2-5	
<i>Lymphatic plasma cells</i>		
Plasmoblasts	0-0 5	
Lymphatic plasma cells (mature)	0-0 3	0 5 0 83
Megakaryocytes	0	0 15-2 0

of individual Splenograms

the latter case the lymphocytes were 5-16 per cent higher Table 3 shows the results of such parallel investigations of 3 cases

All these observations support the view that the qualitative picture obtained by spleen puncture accurately represents the distribution of free cell types in the spleen

We have already pointed out that the reticulum cells of the spleen stroma are found in only small numbers because they form a firm syncytium For this reason far larger numbers of these cells are found in sections than in films as is also the case in the sternal marrow (Rohr) This fact is of no importance for clinical and diagnostic purposes because the significant variations affect the free cells of the organ

The Normal Splenogram The variations of the normal splenograms are shown in Table 4 As only definitely enlarged spleens are punctured and as normal spleens are only rarely removed by operation it is not strictly true to say that the normal splenogram is known Hellmann's histological investigations suggest that the number of lymphocytes in the spleen diminishes with increasing age but our punctures of pathological spleens do not give this impression

For differential diagnosis the most important feature is the

FIG 26a Graphic representation

	Normal values	Normal										Acute inflammatory									
		10	20	30	40	50	60	70	80	90	100	10	20	30	40	50	60	70	80	90	100
Macrophages	0.1-0.3											1									
Plasmacyt retic cells	0.2-0.8	1										1									
Pulp cells	0.2-0.6	1										1									
Tissue mast cells	0-0.1																				
Erythroblasts	0-0.1-0.2	1																			
Myeloblasts	0																				
Myelocytes immature	0																				
Myelocytes $\frac{1}{2}$ mature	(+)																				
Myelocytes mature	0.05-0.1																				
Metamyelocytes	0-0.1																				
Neutrophils staff	1.0-7.0																				
Neutrophils segmented	8-25																				
Eosinophiles mature	0.2-1.5	1										1									
Basophiles mature	0.1-1.1	1										1									
Monocytes	1.2-2.4																				
Lymphoblasts	0-0.2																				
Lymphocytes sm yng	0.6-7.5																				
Lymphocytes large yng	0.4-3.0																				
Lymphocytes sm old	55-79.5																				
Lymphocytes large old	2-5																				
Plasmoblasts	0-0.05																				
Lymphatic plasma cells mature	0-2.3																				
Megakaryocytes	0																				

We were surprised at the slight variations of the values and it is justifiable to draw the conclusion that splenograms obtained by spleen puncture give a relatively accurate picture of the cellular composition of the spleen but, of course only if there is no considerable admixture of blood. Thus as in the case of myelograms the findings can be directly compared with one another.

We have expressed all the values as percentages which are based on examination of at least 1000 cells. In the case of myelograms Rohr enumerates the white cells separately and then mentions the number of reticulum cells and erythroblasts seen while counting each 100 white cells. This method has the advantage of permitting the values found for the granular series to be compared with the blood picture. In the spleen where lymphocytes normally preponderate very greatly this method of differentiation serves no purpose and equally it is of no value to indicate the number of other types of cells per 100 lymphocytes.

On a number of occasions we have compared splenograms made from puncture during life and also from films made directly after splenectomy and we were surprised how little they differed from one another. The main difference occurred if the spleen had been compressed during the operation or if the films were made some time after incising the organ (loss of blood from the pulp sinuses). In

through the splenic artery contained 350 000 platelets per c mm while that leaving in the splenic vein contained only 17 000. In 9 cases in which splenectomy was performed he found a distinctly raised lecithin level. This may have played a part in the increased destruction of blood cells.

Many observations seem to support the view that the spleen exerts an hormonal effect on the marrow even although no proof is yet available. Even so the rapid rise of reticulocytes and the emigration of cells from the marrow even during the first few hours after removal of the spleen seems to be best explained by such a mechanism.

Then again the concept of a correlation between lymphatic and myeloid tissues has much to commend it. For instance Miller and his colleagues found a substance in the urine of patients with lymphatic leukaemia which was capable of inhibiting myelopoiesis while the converse was also found to be the case. It would seem reasonable to suppose (Rohr) that great lymphatic hyperplasia in the spleen (as in Felty's syndrome) might in a similar way have an inhibitory action on the marrow. Perhaps the anaemia and granulocytopenia found in some chronic lymphatic leucosis is partly due to the same mechanism and not only to infiltration of the marrow by leukaemic cells because this infiltration may be absent in spite of marked anaemia and granulocytopenia. Certainly the latter is not always demonstrable in anaemic and granulocytopenic cases of lymphatic leukaemia.

Occurrence of Hypersplenism. Hypersplenism can occur in association with any type of splenomegaly and we shall refer to this in the appropriate sections. Here only a list (in order of frequency) will be given —

- (1) Non inflammatory splenomegalies
 - Splenomegalic cirrhosis
 - Thrombosis of splenic vein
 - Hæmolytic anæmias
 - Storage diseases
- (2) Inflammatory diseases of the spleen
 - Chronic malaria
 - Felty's syndrome
 - Splenic tuberculosis
 - Syphilis
 - Kala azar
 - Sarcoidosis (Boeck)
- (3) Neoplastic
 - Lympho sarcoma
 - Sarcoma
 - Primary malignant reticuloses
 - Hodgkin's disease

maximum value of the lymphocytes as well as the maximum values of inflammatory cells viz staff forms polymorphs and monocytes. We have given the figures of Weil and Tempka for comparison with our normal values although the difference of terminology makes this rather difficult.

M. HYPERSPLENISM AND INHIBITION OF THE MARROW

Naegeli stressed the intimate relation between spleen and marrow recognising the condition of hypersplenic inhibition of the marrow which he attributed to an endocrine action of the enlarged spleen. This idea has been accepted by a number of other writers e.g. Rohr, Heilmeyer, Mallarme, Doan, Dameshek, Hittmair etc.

Clinical Features. Hypersplenism is not a disease *sui generis* but is a syndrome. There is always some degree of splenomegaly which is accompanied by a more or less severe normochromic or hyperchromic anemia with well marked granulocytopenia and sometimes by thrombocytopenia. Usually the whole hemopoietic tissue is affected but one component may be more so than the others.

The bone marrow is usually hyperplastic but in the later stages may become hypoplastic. The myelogram shows a shift to the left i.e. a relative increase of immature myelocytes and basophile erythroblasts sometimes with macroblasts and an increase of megakaryocytes some being of young type. In other words marrow puncture reveals a picture of marrow inhibition with delayed cellular maturation i.e. shift towards immaturity and a tendency to the development of large cells. This explains the characters of the blood picture in such cases but occasionally the marrow does not show a leftward shift and it is clear therefore that the idea of an endocrine inhibition of the marrow by the enlarged spleen is not a complete explanation of the hypersplenic syndrome.

Aetiology. There are three possibilities —

- (1) Increased destruction of cells in the enlarged spleen
- (2) Endocrine inhibition (splenic in origin) of the marrow
- (3) Excessive lymphocytopenia in the enlarged spleen and perhaps also in the other enlarged lymphatic tissue with consequent disturbance of the correlation between the lymphatic and the myeloid systems.

It is probable that all three factors are concerned in the genesis of the syndrome. The increased destruction may be due to delay of the cells in the enlarged spleen thus adding to the chance of lysis. Thus it is known that the spleen can destroy blood cells in virtue of its possession of hemolysins (see chapter on hemolytic anemias) and it is probable that it can remove leucocytes and platelets by a similar mechanism. Doan described a typical case of the hypersplenic syndrome in which the blood entering the spleen

PART II SPECIAL

THE SPLENOGRAM IN SPLENOMEGALIES

We have discussed the various forms of splenomegaly from a clinical and diagnostic point of view rather than from a strictly pathological one

A NON INFLAMMATORY HYPERPLASIAS OF THE SPLEEN

In this group fall those diseases in which enlargement of the spleen is due to increased functional demand and also to stasis in the portal circulation *e.g.* splenomegaly in hepatic cirrhosis thrombosis of the splenic or portal veins and the hæmolytic anæmias

Splenomegaly due to compensatory revival of hæmopoiesis in the spleen also falls into this group

1 CIRRHOSIS OF THE LIVER

Spleen puncture is only of diagnostic value in those cases in which enlargement of the spleen dominates the clinical picture sometimes for years before hepatic disease is detectable (Heilmeyer)

In such cases differential diagnosis may lie between chronic inflammatory splenomegaly aleukæmic lymphadenosis isolated Hodgkin's disease of the spleen and a storage disorder *e.g.* Gaucher Here spleen puncture will be valuable because the splenogram associated with hepatic cirrhosis unlike that in most other diseases is practically normal

Some degree of splenic enlargement is discoverable in all cases of hepatic cirrhosis although the cause of this is still not known with certainty Sternberg incriminates the same agent that causes the cirrhosis attributing much less importance to stasis in the portal circulation Herxheimer admits the importance of stasis but asserts that there is also genuine hyperplasia of the spleen Probably these are not the only relevant factors For instance the splenomegaly that occurs in Kinnier Wilson's disease suggests the importance of nervous influences while some unknown noxa has to be postulated in those cases in which great enlargement of the spleen precedes the development of hepatic cirrhosis sometimes for years

We have seen the syndrome in association with splenomegalic cirrhosis (p 55) in one case of hemolytic familial jaundice lympho sarcoma and one case of reticulosis (p 184) Wintrobe has recorded an instructive case of the syndrome in association with syphilis of the spleen (see also Curschmann) Cartwright has discussed its occurrence in kala azar Many other references will be found in the relevant literature especially Doan Mallarme and Dameshek

Some degree of marrow inhibition presumably of splenic origin also occurs in chronic myeloid leukemia (see our discussion of this fact in the chapter on leukemias)

Treatment The condition may be so severe as to make treatment imperative especially in the dangerous cases with severe thrombocytopenia The following is a list of the more important indications —

(1) Cure of the infective cause of the splenomegaly *e g*, malaria syphilis kala azar tuberculosis

(2) X irradiation when removal is not possible or the condition is neoplastic *e g*, Hodgkin's disease sarcoma primary reticulosis

(3) Splenectomy

Specific treatment may cure the syndrome *e g* in malaria kala azar and syphilis In primary reticulosis with extreme leucopenia (500-720 per c mm) and an hemorrhagic diathesis we saw great improvement after careful radiotherapy to the spleen (p 185) A similar effect may be obtained in severe thrombocytopenia when splenectomy is contra indicated on account of an acute exacerbation In all other cases splenectomy is the treatment of election

It is particularly in case of hypersplenic inhibition of the marrow that spleen puncture is of the greatest value in diagnosis and in indicating the most suitable line of treatment

for the production of some globulins especially when the increased number of plasmacytoid reticulum cells is combined with a remarkable enlargement of this organ as in splenomegalic cirrhosis and in chronic inflammatory conditions

In the second case there is a combination of alcoholic cirrhosis with a secondary hemolytic anemia (according to Eppinger by no means a rarity). The spleen was enlarged (23 cm) and puncture again revealed an increase of plasmacytoid reticulum cells probably as in the first case attributable to the hepatic changes. The large number of pigment macrophages (1.8 per cent instead of 0.1 per cent) was presumably a consequence of the increased hemolysis while the erythroblastosis (5.8 instead of 0.1 per cent) and the augmentation of the number of myelocytes (1.6 per cent instead of 0.1-0.2 per cent) is usual in hemolytic diseases (p. 69)

2 SPLENOMEGALIC CIRRHOSIS

For clinical purposes it is convenient to distinguish these cases from the typical examples of cirrhosis. Unlike Laennec's cirrhosis which is usually due to alcohol (Wuhrmann and Constam) the aetiology of splenomegalic cirrhosis is unknown but certainly usually occurs at an earlier age (Heilmeyer). Such authorities as Heilmeyer, Weil, Fanconi, etc. believe that a condition worthy of being called Banti's disease does exist although most writers would grant it only the status of a syndrome (Jentzer and Weyeneth).

The term is applicable to primarily chronic splenomegaly possibly of varying aetiology which is often followed by hepatic cirrhosis and in which anemia, leucopenia and thrombocytopenia occur early, i.e. an anemia of Naegeli's type of hypersplenic inhibition of the marrow. Bleeding from the gastro-intestinal tract may precede the development of cirrhosis but splenectomy in the early stages can produce permanent cure (Heilmeyer).

The following is a typical example —

B. F. aged 38 male

History. Vague abdominal discomfort at age 23, appendectomy—operation (Billroth II) a year later but discomfort persisted. In 1940 (age 34) diarrhoea, meteorism and anemia (haemoglobin 59 per cent) with moderate ascites. No history of alcohol or of jaundice. Liver not enlarged. Spleen (7 cm) firm and easily palpable. Haemoglobin 72 per cent, red corpuscles 3,600,000 per c mm, leucocytes 3,000 per c mm, platelets 40,400 per c mm. Thus a typical splenopathic inhibition of the marrow. During the next four years the spleen became progressively larger (23 cm) and there was occasional melæna. The haemoglobin fell to 59 per cent, there was no improvement with liver extracts and only slight effect from iron.

After 3 years (1943) it was decided to perform splenectomy and a Talma operation. There was typical nodular cirrhosis (biopsy). Spleen 750 g, 23 cm. No ascites. The operation was followed by prolonged pyrexia and ascites and pleural effusion gradually developed. Death

1 LAENNEC'S CIRRHOSIS

Table 5 shows the splenograms of 2 cases. The first case was a typical alcoholic cirrhosis (Takata+++ SR 35 bilirubin 1.8 prothrombin 30 per cent) and a definitely palpable spleen (17 cm). The sternal marrow showed a marked increase of plasmacytoid reticulum cells (34 per 100 leucocytes) and the spleen showed a similar increase (4.3 per cent). The normal for inflammatory conditions is only 0.2-0.8 per cent and the highest values in a chronic inflammatory splenomegaly (Felty's syndrome) were 10.3 per cent and 17 per cent. The splenogram also shows an increase of neutrophils and monocytes both of which are probably due to portal stasis and therefore congestion of the red pulp. Pigment macrophages but no other phagocytes were scanty while the pulp cells were somewhat increased.

TABLE 5

Case number	1	2	Case number	1	2
Age Sex	54♂	50♂	Erythroblasts	—	5.8
<i>Hemogram</i>			Myelocytes	0.2	1.6
Leucocytes (in 1 000s)	6.2	3.2	Neutrophiles staff	4.2	5.0
Neutrophiles staff	17	22	Neutrophiles segmented	23.7	3.9
Neutrophiles segmented	50	25	Eosinophiles	2.3	1.4
Eosinophiles	4	4	Basophiles	0.2	0.9
Basophiles	2	1	Monocytes	7.1	1.8
Monocytes	17.1	6.1	Lymphoblasts	—	0.1
Lymphocytes	13	4.1	Lymphocytes small young	4.4	4.7
<i>Splenogram</i>			Lymphocytes small old	47.5	68.5
Macrophages	0.1	1.8	Lymphocytes large young	1.0	0.7
Plasmacyt retic cells	4.3	2.4	Lymphocytes large old	2.3	0.6
Pulp cells	2.5	0.1	Lymphocytes Total	55.2	74.1
			Plasma cells	—	0.8

Rohr, Klima, Fleischhacker, Wuhrmann and others have pointed out the relationship between the raised plasma globulin (according to Wuhrmann and Wunderly who used the electrophoretic method mainly β and γ globulin) and the increase of plasmacytoid reticulum cells. The neoplastic cells of plasmacytomas usually produce γ globulin but only in exceptional cases the β type. Wuhrmann inclines to the view that the dysproteinemia is primary and the increase of plasmacytoid reticulum cells secondary (further details see p. 14). We adhere to the idea of the formation of certain globulin fractions by these cells perhaps however only in the sense of Brass's protein transformation.

In the above case of advanced cirrhosis with strongly positive Takata reaction and greatly increased sedimentation puncture revealed as great an increase of plasmacytoid reticulum cells in the spleen as in the marrow.

If then these elements are admitted to be producers of certain globulins it is to be expected that even the spleen is of importance

TABLE 6b

Case	Macrophages	Plasma cells	Polymorphs	Erythroblasts			Myelocytes	Neutrophils		Eosinophiles	Basophiles	Monocytes	Lymphocytes	Plasma cells	Megakaryocytes	
				Reticular	Polychrom	Orthochro		Stalk forms	Polymorphs						Half mature	Mature
1 47	03	04	08	12	33	09	12	21	79	07	05	20	79	12	—	—
				37				93								
2 37	06	09	26	51	100	02	04	108	281	57	20	42	29	8	++	++
				153				389								
3 39	03	13	03	03			20	130	217	40	03	40	49	6	++	(+)
								347								

In Case 2 a 37 year old woman presented the picture of severe cirrhosis with ascites (plasma bilirubin 2.0 mg per cent Weltmann 0.15 widened prothrombin 50 mg per cent Takata and Cephalin ++). The diagnosis was histologically confirmed. In spleen films up to 15 per cent of basophile macroblasts were found together with distinct neutrophile reaction.

In the third case a man of 39 the clinical features were those of early cirrhosis (Weltmann widened 0.1 Takata and cadmium + prothrombin 60 per cent fibrinogen 0.5 mg per cent) although histological examination of the liver was not possible. The spleen contained a few erythroblasts and numerous megakaryocytes.

These 3 cases might probably show that the presence of erythroblasts and megakaryocytes in spleen puncture are consistent with the presence of inflammatory cirrhosis of the liver (alcoholic origin could be excluded in all 3 cases) if the spleen changes are not due to the occurrence of haemorrhage. Of course more cases are needed for confirmation of these inferences especially for the determination of whether the same agent that causes the cirrhosis also causes the development of the ectopic foci of hemopoiesis. The presence of megakaryocytes is of special interest.

(b) *Schistosomiasis* For the sake of completeness we will mention that in Egyptian splenomegaly the picture may resemble splenomegalic cirrhosis in which thrombosis of the splenic vein may occur in the late stages. Wintrobe asserts that the only distinctive feature is the presence of the parasites in the stools. The disease produced by *schistosomiasis mansoni* or *japonicum* is not only found in the East but also in South and Central America.

occurred 1½ years later. At autopsy: healed (post operative) peritonitis small nodular cirrhotic liver but no thrombosis of portal or splenic veins

TABLE 6a

Date	1/41	11/42	10/43	Date	1/41	11/42	10/43
	Puncture		Film *		Puncture		Film *
Hæmogram				Tissue mast cells	—	—	0.1
Leucocytes (in 1 000s)	3.1	2.9	2.4	Myelocytes	—	0.1	—
Neutrophiles staff	25½	44	35	Neutrophiles staff	6.6	5.8	3.4
Neutrophiles segmented	25	32½	38	Neutrophiles segmented	21.3	5.4	5.8
Eosinophiles	8½	11	6½	Eosinophiles	2.4	2.3	1.5
Basophiles	2½	1	½	Basophiles	0.9	0.3	0.3
Monocytes	5½	10½	5½	Monocytes	3.8	2.0	2.7
Lymphocytes	33	20½	14½	Lymphoblasts	—	0.2	0.5
Splenogram				Lymphocytes small young	4.0	7.6	8.8
Macrophages	—	—	0.1	Lymphocytes small old	60.1	71.9	64.7
Plasmacyt retic cells	0.2	1.0	0.1	Lymphocytes large young	—	1.0	5.8
Pulp cells	0.2	1.0	0.4	Lymphocytes large old	2.5	1.4	5.5
				Lymphocytes Total	64.6	81.9	85.3
				Plasma cells	—	0.7	0.3

After splenectomy

This is clearly a case of splenomegalic cirrhosis which at first simulated a case of thrombosis of the splenic vein. The splenogram was normal and inflammatory neoplastic and leucotic conditions could therefore be excluded. Even a later splenogram (1942) was normal indeed the resemblance of the two from *in vivo* punctures and one made from films at operation is very striking (Table 6a). Perhaps the rather raised lymphocyte value can be regarded as more suggestive of splenomegalic cirrhosis than of portal thrombosis possibly being part of the general effect of splenic activity on hæmopoiesis.

Even when the splenogram is normal it is very important to differentiate between splenomegalic cirrhosis and portal thrombosis because the earlier splenectomy is carried out in the former the better the outlook. *e.g.* Heilmeyer has recorded cure in a boy of 15 whereas the expectation of life is otherwise about 7 years. Torrioli and Pusic recorded 4 cases with 2 cures 1 of more than 5 years duration. Perhaps some inflammatory cases may be outlined from this idiopathic form.

(a) *Splenomegalic Cirrhosis of Inflammatory Origin*: In 2 cases liver biopsy revealed the typical appearances of inflammatory cirrhosis with much interstitial inflammation increase of connective tissue proliferation of lymphocytes and plasma cells.

The splenogram is given in Table 6b.

Case 1 was a woman aged 47 who complained of vague abdominal discomfort and whose spleen was distinctly enlarged while the plasma bilirubin was raised to 1.8 mg per cent. Spleen puncture revealed 3.7 per cent erythroblasts and occasional myelocytes but no megakaryocytes.

numerous varicosities in the lower third. Serum iron 22 gamma per cent. Prothrombin concentration reduced = 45 improvement after administration of iron and blood transfusion. Operation was refused and the patient remained well until January 1944 when there was again severe bleeding. The spleen was now 20×14 cm. smooth and hard with little movement on respiration. Liver not enlarged. No collateral circulation observable on the abdominal wall. Haemoglobin 33 per cent. red corpuscles 2 300 000. reticulocytes 10.3 per cent. leucocytes 2 600 with 8 per cent lymphocytes. platelets 18 000. cholesterol reduced 56. serum iron 30. Takata negative. bilirubin 0.6. protein not reduced = 6.7. prothrombin 40 mg per cent.

Oesophageal varices were very well marked but no radiographic changes in the stomach.

Sternal puncture showed increased erythropoiesis with slight general immaturity (Spleen puncture see Table 8). Blood transfusion and

TABLE 8

Date	7/44	3/44	Date	2/44	3/44
	Punc- ture	Film		Punc- ture	Film
<i>Haemogram</i>			Myelocytes	0.3	0.2
Leucocytes (in 1 000s)	28	33	Neutrophiles staff	7.0	4.0
Neutrophiles, staff	16	11	Neutrophiles segmented	72.2	12.9
Neutrophiles segmented	43	56	Eosinophiles	7.3	2.3
Eosinophiles	3	2	Basophiles	0.6	0.2
Basophiles	3	—	Monocytes	4.1	2.7
Monocytes	12	7	Lymphoblasts	0.1	0.1
Lymphocytes	22	22	Lymphocytes small young	1.6	10.5
			Lymphocytes small old	53.7	64.1
<i>Splenogram</i>			Lymphocytes large young	0.3	0.8
Plasmacyt retic cells	0.9	0.3	Lymphocytes large old	2.7	1.1
Pulp cells	1.8	0.5	Lymphocytes Total	60.4	76.6
Erythroblasts	—	0.1	Plasma cells	0.4	0

administration of iron raised the haemoglobin to 85 per cent. A diagnosis of thrombosis of the splenic vein was made.

Operation was performed in the Surgical Clinic of Professor Brunner. On March 7th 1944 the spleen 21×12 cm. weighed 750 g. There was a mass of veins as thick as the thumb at the upper pole of the spleen and collateral veins as thick as a pencil running to the diaphragm. Removal of the spleen was difficult because of numerous adhesions and the exact condition of the splenic vein could therefore not be determined but there was no thrombosis in the hilum. Liver and portal veins appeared normal.

Histological examination of the spleen in the Pathological Institute in Zurich (Professor v. Meyenburg) showed dilated sinuses similar to the condition seen in portal stasis but no other abnormalities were detected.

In the autumn of 1944 the general condition was good. haemoglobin 90 per cent. leucocytes 8 400. platelets 120 000. occasional Howell-Jolly bodies in the red corpuscles such as are usually found after splenectomy. The oesophageal varices were distinctly smaller but were still detectable.

This case shows a typical picture of thrombosis of the splenic

3 WILSON'S DISEASE

Very large spleens may be found in this condition (Klemperer) but at this stage cirrhosis and other clinical features are fully developed and spleen puncture is of no diagnostic importance. In a typical case in a 30 year old man with ascites and a large spleen puncture showed the following splenogram (Table 7)

TABLE 7

Haemogram								Splenogram											
Total leucocytes (in 1 000's)	Neutrophiles staff	Neutrophiles segmented	Eosinophiles	Basophiles	Monocytes	Lymphocytes	Plasma cells	Plasmacytoid retic cells	Pulp cells	Fat cells	Neutrophiles staffs	Neutrophile segmented	Eosinophiles	Basophiles	Monocytes	Lymphocytes small	Lymphocytes large	Lymphocytes Total	Plasma cells
28	13	46	6	1½	10	23	1	0.7	2.5	0.1	6.9	22.0	8.1	0.9	3.6	54.3	0.5	54.8	0.4

There was well marked eosinophilia (8.1 per cent as compared with the normal 0.2-1.5 per cent) which was also noticeable in the blood (6 per cent). Death occurred a year later in spite of a Talma operation. Autopsy confirmed the diagnosis but did not reveal any noteworthy histological changes in the spleen (740 g)

4 HÆMOCHROMATOSES

In one case pigment macrophages were very numerous but as there was a good deal of blood mixed with the spleen juice the percentage could not be evaluated. In one case of secondary hæmo-chromatosis with splenomegaly which occurred after many blood transfusions in a case of aplastic anaemia hæmosiderin macrophages were numerous (0.6 per cent) (Moeschlin and Rohr 8)

II THROMBOSIS OF THE SPLENIC AND PORTAL VEINS

Eppinger has paid much attention to the thrombosis of the splenic vein the symptoms of which were clearly described by Naegeli (2)

We have details of 3 cases —

Case 1 B.G. aged 21 female shop assistant

At the age of 9 suspected appendicitis—no operation but at the age of 18 (1941) sudden severe gastro intestinal hæmorrhage

The spleen was enlarged (11 cm) but the liver was not. Hæmoglobin 45 per cent red corpuscles 2 600 000 per c mm leucocytes 2 300 per c mm platelets 47 000 X ray examination of the œsophagus showed

reactive formation of extramedullary foci can be so great as to produce distinct enlargement of the spleen. Probably the immature cells never belong to one series only—granulocytes and erythropoietic elements occur together although one may predominate. The following types can therefore be distinguished—

(1) Mixed myelocytic erythroblastic types

- (a) Compensatory
- (b) Chronic inflammatory
- (c) Leukæmic

(2) Predominantly erythroblastic types

(3) Cases with numerous megakaryocytes

(1) Mixed Myelocytic Erythroblastic Types Splenomegaly which is mainly due to the presence of extra medullary hæmopoietic activity can occur as a compensatory phenomenon in association with all those conditions in which the marrow is extremely reduced in amount but also in chronic inflammatory states and in leukæmias where it may be neoplastic.

(a) Compensatory Types Here a typical example is found in marble bone disease so called osteosclerotic anemia in which the bone marrow is progressively reduced in amount and ultimately almost destroyed by osteosclerosis. Extra medullary foci of blood formation develop in the liver and in the spleen often with considerable increase in size of both organs (Naegeli). Similar changes may occur with extensive deposits of tumour in the bone marrow e.g. carcinomatosis sarcomatosis and multiple myeloma. We saw a case of carcinoma of the breast with extensive metastases in the marrow of many bones in which as a result of extensive myeloid metaplasia the spleen was enlarged (15 × 9 cm). In the blood there were 15 500 erythroblasts per c mm and many myelocytes.

Difficulties in differential diagnosis may only arise in cases of osteomyelosclerosis and osteomyelofibrosis (see p. 64).

(b) Chronic Inflammatory Types In association with inflammatory stimuli some immature blood cells can always be found in the spleen—myelocytes usually being more numerous than erythroblasts while megakaryocytes are rather uncommon. As compared with the leukæmias the increase of myelocytes and erythroblasts is relatively small the former varying between 0.1 and 1.7 per cent and the latter between 0.2 per cent. Such changes are much commoner in definitely chronic cases in which occasionally numbers as high as 9–15 per cent are found.

Occasionally the splenomegaly and the appearance of immature cells in the blood may lead to confusion with leukæmia as in the following case—

H. J. aged 65 male

Had suffered from polyarthritides for 10 years. In 1945 the spleen was found to extend to the navel. In March 1943 in Professor Rossier's

vein with a persistent hard swelling of the spleen extending over many years and repeated gastro intestinal bleeding. In the absence of any signs of liver disease. Operation did not reveal any phlebitis in the splenic hilum but the greatly dilated veins and the numerous collaterals to the superior vena cava, together with the absence of signs of portal stasis indicate that there was a mechanical obstruction somewhere in the area drained by the splenic vein.

During the first splenic puncture blood ran into the syringe no doubt as a result of congestion and it was only when a second puncture was performed with a minimum of suction that useful films were obtained and from these a splenogram was made and for comparison a second one was made from films obtained from the spleen at operation (Table 8).

The only change is slight increase of pulp cells such as has been found in all chronic inflammatory splenomegalies. The plasmacytoid reticulum cells, staff forms and monocytes are not increased and an inflammatory condition can therefore be excluded with certainty this was later confirmed by histological examination. In films from the spleen removed at operation there are rather more lymphocytes and fewer neutrophils probably because blood had run out of the dilated sinuses.

In another unusual case thrombosis of the portal and splenic veins had spread from a varicose dilatation in the liver. The splenogram was normal except for a slight increase of neutrophils probably due to admixture of blood on account of stasis in the spleen. A third case also presented a normal splenogram.

It seems clear therefore that a normal splenogram associated with well marked splenomegaly and the typical picture of splenopathic inhibition of the marrow strongly suggests splenomegalic cirrhosis or if the clinical features are consistent a chronic splenic or portal vein thrombosis. Perhaps raised lymphocyte values of 80-85 per cent are rather more in favour of splenomegalic cirrhosis. The presence of a definite inflammatory reaction is of course suggestive of an inflammatory cirrhosis. If a puncture reveals a distinct increase of plasmacytoid reticulum cells in the absence of signs of inflammation the evidence in favour of cirrhosis is fairly strong.

III SPLENOMEGALY WITH ECTOPIC FOCI OF BLOOD FORMATION

In this section some indications of the differential significance of distinct increase of immature myeloid cells in spleen puncture will be given. Further details will be found in the relative sections.

It has already been said that a few myelocytes and less commonly a few erythroblasts may be found in spleen puncture in chronic inflammatory conditions. In some circumstances compensatory or

myeloid leukaemia but the toxic granules of the neutrophils and the practically normal number of eosinophils were rather against this view. Sternal puncture showed distinct immaturity of myelopoiesis but not sufficient to make a definite diagnosis of leukaemia. It was not until spleen puncture was done that the problem was solved. This showed a typical inflammatory picture with a high percentage of neutrophils (31 per cent) distinct increase of plasmacytoid reticulum cells (3 per cent) and slight increase of myelocytes and erythroblasts. The latter were not as numerous as in cases of myeloid leukaemia in which we have found myelocytes to be between 20 and 60 per cent and the erythroblasts between 1 and 35 per cent except in erythroleukaemias where they may be as high as 59 per cent. The low lymphocyte value (57 per cent) in the present case excluded aleukaemic lymphatic leukaemia as the cause of the great splenomegaly.

Autopsy confirmed the diagnosis of a chronic inflammatory splenomegaly. Probably this was an example of the rare splenic enlargement that may accompany chronic rheumatic polyarthritis (Veil (2)) which probably belongs to the group known as Still's disease. The carcinoma of the oesophagus was not related to the enlarged spleen which was already noticeable long before any signs of the neoplasm developed.

(c) *Leukaemic Types* The leukaemias are even quantitatively distinguishable by spleen puncture because of the much greater numbers of immature cells: thus myelocytes in our observations varied between 8 per cent after arsenic or X ray treatment up to 60 per cent (Table 22) in a fully developed stage. There are of course morphological abnormalities in the leukaemic cells (see p. 130).

(2) *Predominantly Erythroblastic Types* The number of erythroblasts in spleen puncture is highest in four conditions: viz. haemolytic anaemias 0.2-5.8 per cent, pernicious anaemia during relapse up to 13 per cent, myelosclerosis and osteosclerosis (5-20 per cent), leukaemias (1-35 per cent) especially the so called erythroleukaemias (up to 59 per cent in one of our cases).

(3) *Cases with Numerous Megakaryocytes* We found occasional megakaryocytes in chronic inflammatory states e.g. in spleen puncture of a case of undulant fever in which haemolytic anaemia occurred. Presumably the combination of the two morbid conditions were the cause of the extensive extramedullary blood formation. In the foci erythroblasts were numerous (9.3 per cent) and myelocytes were found in each film.

In inflammatory splenomegalic cirrhosis (see p. 56) megakaryocytes were also numerous and were associated with up to 15 per cent erythroblasts.

Megakaryocytes may be scanty or numerous in the leukaemias and also in osteosclerosis. Lang has called attention to their pre-

Clinic persistent leucocytosis of 12 000 to 14 000, with a few myelocytes was discovered. The blood picture on March 19th, 1943 was red corpuscles 3 700 000 leucocytes 13 900 half mature myelocytes 1.5 per cent staff forms 6.5 per cent segmented polymorphs 66.5 per cent eosinophiles 2 per cent basophiles 3.5 per cent monocytes 4 per cent lymphocytes 16.0 per cent platelets 65,000 per c mm.

The granules of the neutrophiles were rather larger than normal and the nuclei of some of these cells were pyknotic. A year later after a period in which dysphagia had become troublesome he was admitted to the Clinic (May 1944).

There was distinct cachexia the spleen was 15 x 11 cm easily palpable and hard the liver and lymphatic glands not enlarged. Red corpuscles 4 800 000 per c mm haemoglobin 99 per cent, colour index 1.03 reticulocytes 2.1 leucocytes 29 700 mature myelocytes 0.5 staff forms 19 segmented polymorphs 70 eosinophiles 1.5 basophiles + monocytes 5.5 lymphocytes 3.5 platelets 267 000. The neutrophile granules were rather coarse and tended to show slight basophilia. Sternal puncture showed erythroblasts 46 immature myelocytes 5 half mature myelocytes 9 mature myelocytes 3.7 metamyelocytes 1.0 staff forms 39 segmented polymorphs 17.7 eosinophiles 2.3 monocytes 0.3 lymphocytes 23.0. The splenogram is shown in Table 9a.

Radiography revealed the presence of a carcinoma of the oesophagus. Death occurred on May 25th 1944.

The autopsy showed advanced chronic inflammatory splenomegaly the organ weighing 850 g and measuring 23 x 12 cm. Histologically the trabeculae were thickened the follicles very small and the pulp extremely cellular with many pulp cells. Haemosiderin macrophage lymphocytes many neutrophiles a few eosinophiles and an occasional myelocyte were present. Plasma cells were scanty. The liver showed slight polymorph infiltration of Glisson's capsule but no round cell infiltration. The carcinoma of the oesophagus was confirmed but no metastases were found.

TABLE 9a

Puncture	1	2	Puncture	1	2
<i>Hamogram</i>			Erythroblasts polychromatic	1.4	0.7
Leucocytes (in 1 000s)	29.7	—	Erythroblasts orthochromatic	0.2	0.4
Myelocytes	↓	—	Erythroblasts Total	1.8	1.1
Neutrophiles staffs	19	—	Myelocytes immature	—	0.1
Neutrophiles segmented	70	—	Myelocytes half mature	0.1	0.4
Eosinophiles	↓	—	Myelocytes metamyelocytes	0.2	0.3
Basophiles	—	—	Myelocytes Total	0.3	0.8
Monocytes	5.5	—	Neutrophiles staffs	7.2	6.7
Lymphocytes	3.5	—	Neutrophiles segmented	23.9	24.3
<i>Splenogram</i>			Eosinophiles	0.1	0.1
Macrophages	0.9	0.9	Basophiles	0.2	0.4
Plasmacyt retic cells	3.0	2.9	Monocytes	4.0	4.7
Pulp cells	0.6	0.2	Lymphoblasts	0.1	0.1
Tissue mast cells	0.1	—	Lymphocytes small	54.0	51.2
Erythroblasts basophilic	0.2	—	Lymphocytes large	3.5	6.5
			Lymphocytes Total	57.6	57.8
			Plasma cells	0.3	0.1

The large spleen and the persistently high leucocyte count with some myelocytes naturally first suggested a diagnosis of chronic

process can lead to osteosclerosis. The typical overgrowth of the marrow may pass over into hypoplasia perhaps on account of the splenic hyperplasia when the sclerosis would be secondary to atrophy of the marrow. On the other hand it might be supposed that the stimulus that causes the proliferation in chronic leukaemia may equally affect the related osteoblasts.

Secondary sclerosis may occur in aplastic anaemia (Bock, Wintrobe). Possibly some of these cases are due to a genuine chronic myelitis as Rohr has suggested. Certainly similar pictures may occur as a result of damage to the marrow by chemical or physical agents e.g. fluorides, strontium, radio active substances, gold etc (Bock).

The blood picture in osteosclerotic anaemia does not show much reduction of red corpuscles or haemoglobin until quite late. The number of granulocytes is usually slightly increased except in the type that develops secondary to aplastic anaemia, then the lymphocytes are relatively increased. There are always some erythroblasts and myelocytes, both of which may be scanty or extremely numerous (Table 9b). In Müller's case which was proved by autopsy, death followed irradiation of the spleen (which clearly indicates that it was not a case of leucosis). There were 15 100 leucocytes with 27.5 per cent of myelocytes and 16.5 per cent of lymphocytes, while there were 4 erythroblasts per 100 white cells.

The spleen is often very considerably enlarged and is fairly hard. Extramedullary myelopoiesis is well marked with many erythroblasts, myelocytes and megakaryocytes, but unlike the condition in myeloid leukaemia the lymphatic follicles are well preserved (Fell, Assman). Undoubtedly some cases belonging to this group have been published as megakaryocytic or aleukemic chronic leucosis.

No previous reports on splenograms of such cases are to be found. Dr Sandkuhler of the University Medical Clinic Heidelberg and Professor Waldenström of the Medical Clinic Uppsala have kindly supplied us with the following spleen punctures on the typical cases of osteosclerotic anaemia (Table 9b). Case 1 which will be published in more detail (Sandkuhler) was one of a woman aged 50 who has suffered from abdominal distension for 13 years. On admission to the Clinic her spleen extended into the pelvis. The blood picture showed anaemia and with up to 44 per cent of immature myeloid cells and about 12 erythroblasts per 100 leucocytes. The bones showed the typical radiographic changes and numerous attempts at marrow puncture failed to supply any marrow. Even trephining of the upper end of the tibia was unsuccessful.

The second case (Professor Waldenström) is probably one of myelosclerosis, details of which have been published elsewhere (Moeschlin 25).

The patient, a woman of 60, was admitted to hospital in 1937 with arthrosis of the hip and mild anaemia (72 per cent haemoglobin, 5 200

sence in polycythemia and they are also present in splenic foci of blood formation in conditions in which the marrow space is diminished e.g. osteosclerosis (Schwartz) carcinomatosis of the marrow (personal observations) and in portal or splenic vein thrombosis (Bonner 1884 Eppinger 1920 Klemper 1928)

In films from the spleen of 2 cases of severe thrombocytopenia (removal by operation) megakaryocytes were fairly plentiful. In both cases the cause was probably of an infective or toxic nature. In the one case there was nephrosclerosis and in the other tuberculous of the spleen and liver (p. 77 and Fig. 28)

IV OSTEOSCLEROTIC AND MYELOSCLEROTIC ANÆMIAS

The various conditions that lead to osteosclerotic anæmia (myelosclerosis leuco erythroblastic anæmia etc.) have at least one feature in common—diminution of the medullary space by osteosclerosis or myelofibrosis. Clinically the spleen may become enlarged as a result of compensatory development of hæmopoietic foci in it while a similar change may occur in some lymphatic glands. Immature myeloid cells appear in the blood but in many cases splenic hæmopoiesis fully compensates for the reduction of the marrow sometimes for years.

Marble bone disease, an inherited malady which manifests itself in childhood belongs in this group (*vide* Naegeli Lang Heilmeyer Rosenthal), but there is a number of apparently allied but ill defined conditions.

There are examples of primary increase of bony trabeculae (diffuse or focal spongiosclerosis) and even of the compact bone but secondary sclerosis is also recognised. Thus fibrosis of the marrow may occur in the leukæmias and aplastic anæmia. Our view is that primary osteosclerosis if it exists is very rare as compared with the secondary type.

Tischendorf and Naumann (4) did not find any examples in their series of leukæmias but we have seen 3 cases (3 typical chronic myeloid leukæmias and 1 erythro leukæmia). In these marrow puncture in the early stages revealed a very cellular marrow, while 2 or 3 years later puncture of the marrow of various bones failed to supply any myeloid tissue. The bones also presented the radiographic signs of increased density and the unusual hardness was very noticeable when punctures were attempted. Storti's cases manifested similar features and Bernard speaks of osteosclerose post leucosique as a late occurrence in some leukæmias. It is quite likely that many published cases of osteosclerotic anæmia were really of this type.

It is particularly interesting that an old standing leukæmic

leucocytes with a normal differential count) In 1943 she developed a normochromic anemia with hæmoglobin 55 per cent leucocytes 5200 premyelocytes 11 per cent myelocytes 7 per cent meta myelocytes 9 per cent and 25 erythroblasts per 100 leucocytes The spleen was distinctly enlarged (14/18 cm) and a number of bone punctures failed to supply any marrow In 1948 the hæmoglobin had fallen to 38 per cent and spleen puncture gave the results shown in Table 9b (Case 2) while sternal puncture only revealed a very hypocellular and oedematous jelly like marrow

Case 3 (Table 9b) a typical myelofibrosis will be published in detail elsewhere

Published reports on the pathological anatomy of the spleen in osteosclerosis and myelosclerosis show that predominance of lymphocytes in spleen puncture corresponds with persistence of the lymph follicles (see the cases of Reich and Rumsey Jackson Parker and Lemon etc)

There are a number of points of interest in these splenograms Thus if similar findings occur in other cases it ought in future to be possible to separate osteosclerotic anemia from the secondary osteosclerosis of chronic myeloid leukemia In splenograms of untreated cases of the latter disease the granulocytes were always between 75 and 97 per cent and even in erythro leukemias if the erythroblasts and granulocytes are taken together the figures are similar while there is also distinct reduction of lymphocytes (15-20 per cent) Treatment with arsenic X rays urethane etc naturally changes these relationships considerably inasmuch as the immature cells diminish and the mature ones increase while the lymphocytes approximately double themselves (Table 22 cases 5 6 8)

In osteosclerotic anemia on the other hand the percentage of lymphocytes is still high (up to 60 per cent) with a relatively small number of immature cells (myelocytes erythroblasts and occasional megakaryocytes) amounting to about 25-35 per cent *This in spite of the intense extramedullary blood formation; the spleen still retains much of its original lymphaderoid structure whereas in the leukemias this is totally obliterated and the tissue much more closely resembles bone marrow*

In most cases of chronic myeloid leukemia the immature granulocytes also present signs of disturbance of development in the nuclei and cytoplasm whereas such changes are not found in the non leukemic forms of extramedullary blood formation The occurrence of megakaryocytes in spleen puncture is not pathognomonic of osteosclerosis as they can also be numerous in cases of leukemia (Figs 46 and 49)

A brief note on a case of osteosclerotic leucosis (see Case 4 Fig 26 b) in which we were able to perform a spleen puncture by the courtesy of Professor Waldenström will be given here (for further details see Moeschlin 25)

SPLEEN PUNCTURE

TABLE 9b

Splenogram													
	Total leucocytes in the blood	Plasmacytoid reticulum cells	Erythroblasts		Myeloblasts	Myelocytes				Neutrophils		Lymphocytes	Megakaryocytes
			Metaphilic	Poly chromatic Orthochromatic		Immature	Half mature	Mature	Meta	Stalk Segmented	Monocytes		
Case 1 50 ♀	5600	0.3	—	43 10	—	07 27	93 103			73 33	17	590	+
				53		230				106			
Case 2 60 ♀	5200	0.6	60	122 12	—	—	40 40	70		36 08	—	604	+
				394		150				44			
Case 3 48 ♀	6800	0.6	02	66 82	—	06 16	30 40			84 108	—	514	+
				170		92				19			
Case 4 37 ♂	4400	0.2	20	138 04	14	08 62	134 216			78 30	—	194	—
				162		420				108			

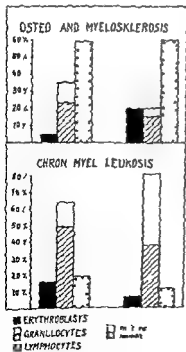


Fig. 46 b Comparison of the splinogram in compensatory extra medullary myeloid metaplasia of the spleen in osteo- and myelosclerosis with the spleen in chronic untreated myeloid leukaemia. In the former there are still 50-60 per cent of lymphocytes (Cases 1 and 2 above) while in the latter there are only 5-25 per cent of lymphocytes. Case 3 was a severe osteosclerotic leucosis with a terminal para myeloblastic exacerbation while Case 4 was an uncomplicated chronic leucosis.

In compensatory extra medullary myelopoiesis in the spleen the fundamental lymphatic structure of the organ remains more or less intact whereas in neoplastic leukemic infiltration this disappears completely.

Another point should be mentioned here. In some cases of myelofibrosis we may be dealing with the subject of a toxic (gold etc.) or inflammatory allergic damage to the marrow.

Experience has shown that in such aplastic anæmias there is as a rule no extramedullary myelopoiesis. Probably this is due to damage affecting the whole of the blood forming system so that the reversion of reticulum cells to erythropoiesis and myelopoiesis is inhibited also in the spleen.

In view of the practical therapeutic importance of a careful clarification of the hereby only mentioned question of osteosclerotic anæmia we beg all observers to publish such cases in detail (with hæmogram, histological findings of bone trepanation, splenogram and X ray films of the bones). We hope that the collecting of more detailed observations will give us a clearer picture of this still problematic disease.

V HÆMOLYTIC ANÆMIAS

The spleen may become considerably enlarged in hæmolytic anæmias. In one of our cases of familial congenital icterus the spleen weighed 1.7 kg. On the other hand in many cases of the so-called acquired form the hard spleen is only just palpable or only enlarged to percussion. We have been able to investigate spleen punctures from 7 cases of hæmolytic anæmia. In one this was combined with cirrhosis and in another with infection by *B. Bang*. The splenograms are shown in Table 10a. In every case there was increase of pigment macrophages and a few polychromatic or oxyphilic erythroblasts were always present. The highest values of these was in the case associated with cirrhosis (5.8 per cent) and in the one associated with Bang infection (9.3 per cent). Most of the pigment macrophages contained hæmosiderin in the form of a golden brown pigment which gave a prussian blue reaction. In only one of the uncomplicated cases were the plasma-cytoid reticulum cells increased (2.7 per cent) and also in the one associated with cirrhosis (2.4 per cent). The pulp cells were only increased in the case of Bang infection but often showed early deposition of hæmosiderin. The lymphocytes values were never diminished.

According to Lubarsch, Gripwall and Heilmeyer there are no constant pathological findings. Lubarsch mentions that the amount of hæmosiderin may be small or extremely great. Gripwall, using rapid fixation of spleens removed at operation, found great dilatation of the sinuses, the lining cells of which projected into the lumen. The lymph follicles were small and scanty.

Spleen puncture has not thrown any light on the genesis of the various forms of hæmolytic anæmia. The one feature common to all types is increased blood destruction, whether this be due to a

Case 4 was that of a man of 37 in whom an enlarged spleen and increased number of leucocytes (22 700 with 18.5 immature granulocytes) was first detected in 1945. The condition improved after irradiation of the spleen but in 1946 a hemorrhagic diathesis developed. X rays revealed a severe and progressive osteosclerosis in the whole skeleton. In spite of repeated transfusions the general condition deteriorated and towards the end of life pathological micromyeloblasts were found in the blood and in spleen puncture (see Table 9b Case 3).

Spleen puncture in this case showed only 19.4 per cent of lymphocytes with 76.4 per cent erythroblasts and granulocytes of which about 7.4 per cent were small paramyeloblasts. This was very unlike the other three cases.

The terminal condition *viz.* the appearance of pathological paramyeloblasts (micromyeloblasts) conclusively indicated that the disease was primary a leucosis.

The presence of the severe osteosclerosis which was confirmed at autopsy may be secondary in the sense used by Bernard (osteosclerose post leucosique) but on the other hand it may be that this may be an example of a condition suggested by Rohr *i.e.* a leucosis in which the involvement of the bones as well as of the marrow is attributed to a primary degeneration of the blood forming and bone forming mesenchyme.

Rohr's opinion that the giant cells found in the spleen in these cases and in other leukemic infiltrations are not megakaryocytes was not confirmed by the appearances in our films. These cells were of the typical transitional forms between immature and mature megakaryocytes.

Spleen puncture permits such osteomyelosclerotic leucoses to be differentiated from osteosclerosis in which splenomegaly is due to a compensatory myeloid metaplasia. In osteosclerosis due to leukaemia one finds as in the above case that in the spleen there is a striking reduction of the number of lymphocytes to 10-25 per cent whereas in the compensatory type of splenomegaly the lymphocytes are relatively high viz. 50-60 per cent.

It appears therefore that in doubtful cases (of course in which there has been no treatment) relatively high lymphocyte values (over 50 per cent) and relatively low myelocyte values (20-30 per cent) strongly suggest a true osteosclerosis or myelofibrosis rather than a genuine leukaemia.

It must be emphasised that in such cases removal of the spleen or exposure to X rays is strongly contra indicated because the compensatory site of blood formation would be destroyed. Even small irradiations can prove fatal; thus Hecht-Johansen (1944) saw death occur as a result of agranulocytosis after administration of only 600 r in one of these cases while in Muller's case (1946) death occurred with progressive anaemia, leucopenia and thrombocytopenia.

spleen appears to have shown that the spherocytosis is itself a cause of increased blood destruction because on account of their shape these corpuscles are not able to pass through the delicate reticulum of the pulp and so become separated from the plasma. It is of course possible that the destruction of red corpuscles in some cases of hæmolytic anæmia may be due to direct phagocytosis in the spleen. This factor is often stressed by pathologists (Hartmann) but in spite of careful search in our cases of hæmolytic anæmia we have never seen phagocytosis of red corpuscles by reticulo-endothelial cells. It must be admitted however that we have not had the opportunity of performing a spleen puncture in the very early stage of a hæmolytic crisis. Except in a case of *B. Bang* but without any hæmolytic anæmia we saw one reticulum cell with phagocytosis of erythrocytes. Baumgartner working on experimental anæmias in guinea pigs was able to demonstrate such phagocytosis in red corpuscles in the liver and spleen. It is therefore possible that a similar condition occurs in some human cases and Schubothé recently demonstrated these findings in a case of hæmolytic anæmia due to an excessive amount of agglutinins.

Owren's view that the hæmolytic crises depend upon a hypoplastic phase of erythropoiesis is open to criticism. It is possible that in such cases there has been an inhibition of erythropoiesis as a result of kidney damage due to increased hæmolysis (augmented urea in all his cases) but as Rohr supposes the appearances are more likely to be due to intercurrent infection. The latter could produce an arrest of the erythropoiesis followed by an excessive drop of the number of the erythrocytes in congenital hæmolytic anæmia due to the shorter life span of these cells (12-14 days) compared with a normal individual where an aplastic crisis (normal life span 120 days) causes a less drastic reaction of the erythrocyte number. We have no doubt that in the absence of complications hæmolysis is the essential cause of the crises in acholuric jaundice and we have found some increase of erythroblasts in the sternal marrow at the onset of a crisis with further increases within 2 or 3 days if the cases were not complicated.

This does by no means contradict the occurrence of aplastic crises. Gasser could demonstrate typical aplastic reactions in different allergic conditions and we ourselves saw a permanent disappearance of all erythroblasts (erythroblastopenia) in a case of aplastic anæmia (see p. 73).

It is striking that the stimulus to erythropoiesis in hæmolytic anæmias leads to recurrence of blood formation in the spleen especially when hæmolysis is combined with an infective factor as in the following case —

P. V. male aged 27 whose brother and grandfather suffered from hæmolytic icterus had complained for some years of progressive fatigue

TABLE 10a

	Uncomplicated cases						
Case	1	2	3	4	5	6	7
<i>Hæmogram</i>							
Age sex	24 ♂	50 ♂	22 ♀	44 ♀	25 ♂	21 ♀	27 ♂
Osmotic resistance maximum	0.42	0.30	—	0.30	0.38	0.32	0.32
Osmotic resistance minimum	0.66	0.50	—	0.46	0.54	0.46	0.67
Hæmoglobin (%)	96	59	—	72	85	70	52
Red corpuscles (in millions)	4.990	2.220	—	3.020	3.940	2.940	2.568
Leucocytes (in 1 000s)	8.4	3.2	—	27.8	4.8	3.4	5.0
Erythroblasts (per 200 leucocyte)	—	3	—	1	—	—	3
Reticulocytes (per 1 000)	—	92	—	159	31½	—	113
Neutrophiles staff	11½	22	—	42	28½	24	27
Neutrophiles segmented	59½	25½	—	40½	34	30	19
Eosinophiles	1½	4	—	1	2	6½	½
Basophiles	—	1	—	½	1	2½	—
Monocytes	9	6½	—	3½	5½	14	8½
Lymphocytes	18	41	—	10½	29	23	44½
<i>Splenogram</i>							
Macrophages	0.3	1.8	2.3	+	—	0.6	0.5
Plasmacyt retic cells	—	2.4	2.7	+	0.2	0.7	0.4
Pulp cells	—	0.1	0.4	+	0.5	0.4	2.2
Erythroblasts basophilic	—	0.6	—	—	—	—	1.9
Erythroblasts polychromatic	—	3.9	0.1	—	—	—	5.8
Erythroblasts orthochromatic	—	1.3	0.1	—	0.1	—	1.6
Myelocytes mature	—	0.7	0.3	0.2	—	—	0.6
Myelocytes half mature	—	0.7	0.2	0.3	—	—	0.1
Myelocytes immature	—	0.2	—	—	—	—	—
Neutrophiles staff	3.3	5.0	1.1	0.8	3.0	6.5	7.6
Neutrophiles segmented	16.3	3.9	11.1	6.4	26.6	9.0	7.5
Eosinophiles	0.9	1.4	1.0	0.6	0.4	1.8	1.2
Basophiles	0.1	0.5	0.3	0.4	0.4	0.1	1.5
Monocytes	1.9	1.8	2.0	2.0	3.0	3.4	3.6
Lymphoblasts	—	0.1	—	0.6	—	0.8	—
Lymphocytes small young	7.5	4.2	2.6	10.0	1.9	7.5	2.3
Lymphocytes small old	61.5	68.5	74.2	66.3	58.7	56.3	61.3
Lymphocytes large young	3.0	0.7	0.3	6.1	0.6	6.3	0.3
Lymphocytes large old	4.9	0.6	0.9	5.8	2.6	5.7	1.4
Lymphocytes total	76.9	74.1	78.0	89.8	65.8	76.6	64.4
Plasmoblasts	—	—	—	0.1	—	0.5	—
Plasma cells half mature and mature	0.3	0.8	0.1	0.4	—	0.4	—

diminished life span of the red corpuscles (as demonstrated in transfusion experiments by Dacie, Ludin and Owren) in congenital familial cases or to formation of auto hæmolysins, auto-agglutinins or perhaps to lysolecithin (de Vries).

The investigations of Gripwall, Dameshek and Schwartz indicate that the part played by the spleen is of the nature of an exaggeration of its normal function and that there is increased spherocytosis as a result of the action of hæmolysins while the corpuscles pass through the organ. Probably the older and less resistant corpuscles are destroyed by these hæmolysins and then the reticulo-endothelium of the spleen with that of other organs removes the debris.

Bjorkmann, who has investigated the minute structure of the

foci in the spleen and this supports Rohr's view that these erythroblasts are not derived from the closed circulation of the bone marrow but enter the circulation from the open vascular system of the spleen and perhaps the liver.

It is interesting that the infective stimulus evoked not only the formation of many erythroblasts and some myelocytes in the spleen but also the formation of considerable numbers of megakaryocytes. This demonstrates clearly that such foci arise from metaplasia, not from emigration from the bone marrow. Megakaryocytes may be difficult or impossible to recognise in histological sections especially in the case of the young types in which the nucleus is not lobulated; they are then likely to be classified as unspecific giant cells. It is only in films that the morphological details can be detected. The occasional myelocytes found in the peripheral blood especially during hæmolytic crises are also derived from extramedullary foci which are present in the spleen in most cases of hæmolytic anæmia.

Numerous hæmosiderin macrophages were found in spleen puncture of a peculiar case of aplastic anæmia which was kept alive for 3 years by repeated blood transfusions although no erythroblasts could be found at all in the marrow (Table 10a, case 6). This case was described in more detail by Moeschlin and Rohr (8). Heilmeyer (1942) and Begemann (1947) have reported similar cases and I have seen a similar one in which erythroblasts were absent from the marrow for at least 6 years (in the clinic of Professor Hansen in Oslo).

Such secondary hæmochromatosis must be due to the fact that the body is unable to make use of the excessive amount of iron derived from the transfused red corpuscles because erythropoiesis has ceased and the metal which cannot be eliminated is therefore gradually accumulated in the reticulo endothelium.

Spleen puncture plays no part in the diagnosis of a typical case of hæmolytic anæmia: the increased reticulocytes, the raised serum bilirubin and the numerous erythroblasts in the sternal marrow enable a rapid diagnosis to be made. In cases where the fragility of the red corpuscles is not increased and when during remission the only clinical sign is splenomegaly, the presence of numerous hæmosiderin macrophages in spleen puncture may arouse a suspicion of latent hæmolytic anæmia especially if in the absence of inflammatory changes some erythroblasts are present.

VI PERNICIOUS ANÆMIA

Splenomegaly is rarely pronounced in pernicious anæmia in adults but in younger patients may be quite considerable (Naegeli). Histologically the spleen shows well marked hæmosiderosis and numerous foci of blood formation (Lubarsch).

severe sweating and occasional slight jaundice. On examination nutrition was good there was no fever a typical tower skull a high palate and a large hard smooth spleen (30 × 16 cm). The liver was also palpable. The blood showed hemoglobin 44 per cent red corpuscles 2.6 mill colour index 0.83 spherocytes and polychromasia. The reticulocytes were 3.7 per cent but at a later examination 11.5 per cent. Leucocytes 5000 per cmm with 34 per cent lymphocytes. The osmotic resistance was greatly diminished the maximum being 0.58 and the minimum 0.40. H.S.R. 56 serum iron 120-170 bilirubin occasionally increased to 2.1.

Sternal puncture showed 240 erythroblasts (basophiles 7.0 poly chromatic 2.10 oxyphilic 2.3) to 100 white cells.

Splenectomy was performed and the spleen was examined in the Pathological Institute at Zurich (Professor v. Meyenburg) it weighed 1.7 kg and was 25 × 15 × 7 cm the capsule was smooth and on section the tissue was dark bluish red. Histologically the follicles were fairly large there were epithelioid cells forming avascular nodules and in the outer zones of the follicles tuberculoid nodules were found in places. The reticulum of the pulp cells was loose but with patchy thickenings and many foci of collagenous fibres and innumerable foci of epithelioid cells were present. There were also infiltrations of the splenic veins. The pulp was infiltrated with leucocytes plasma cells and occasional eosinophiles while in the vicinity of the vessels there were many haemopoietic foci containing erythroblasts with small nuclei and a few large basophilic erythroblasts.

Agglutination of *Bacillus Bang* was positive 1640 the haemogram and splenogram are given in Table 10a. Case 7.

This was an example of typical familial hemolytic anemia with

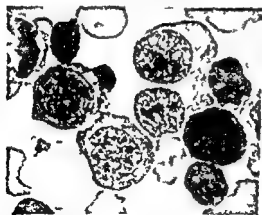


FIG. 27 a

(a) Large basophilic erythroblasts (macroblasts) in a case of hemolytic anemia complicated by chronic inflammatory splenomegaly (Bang).

a strikingly large spleen in which histological examination showed the presence of typical Bang granulomata. This case was described in collaboration with Löffler in an earlier publication on undulant fever.

It must presumably be assumed that the hemolytic anemia was aggravated by the further increase of the splenomegaly due to the infection with *B. Bang*.

Films from the spleen showed a remarkable degree of erythropoiesis (9.3 per cent) with a con-

siderable number of mitoses in the erythroblasts and many large macroblasts some being extremely young (Fig. 27 a).

The presence of numerous erythroblasts in the peripheral blood (75 per cmm) was probably due to the numerous hemopoietic

plasma stained by May Grunwald Giemsa corresponds to these bodies which probably are a special form of mitochondria (see Fig 27 c)

TABLE 10b

Myelo-gram	Splenogram										
Megaloblasts	Plasmacyt retic cells	Pulp cells	Megaloblasts			Myelocytes		Neutrophiles		Eosinophiles	Lymphocytes
			Basophil	Polychrom	Oxyphil	Half mature	Mature	Staff forms	Segmented		
57 0	2 4	0 6	2 4	10 6	0 6	2 2	3 6	4 6	11 0		
			13 6			5 8		15 6		3 4	53 8

A number of authors (Nagy 1924 Introzzi 1929 and Weil 1936) agree that megaloblasts as well as myelocytes are found in spleen films

Since the introduction of sternal puncture spleen puncture has ceased to be of diagnostic value in pernicious anaemia but it does demonstrate that in this disease there is a general disturbance of blood formation which leads to the development of such abnormal elements as megaloblasts even in extramedullary erythropoietic foci. It also demonstrates that the development of megaloblasts does not depend upon some local peculiarity but only upon deficiency of a haemopoietic factor and that in its absence megaloblasts can arise from the reticulum cells of the spleen.

In our view these findings are also proof that megaloblasts in the circulating blood in pernicious anaemia are not derived from the bone marrow but from extramedullary foci mainly in the spleen.

VII THROMBOCYTOPENIA WITH SPLENOMEGALY

It is recognised that the spleen plays a considerable part in the genesis of many forms of thrombocytopenia (Naegeli, Rohr, Heilmeyer). At least two processes are at work viz inhibition of maturation of megakaryocytes in the marrow probably as the result

In a man of 67 with typical pernicious anemia (hemoglobin 37 per cent and red corpuscles 1.2 mill.) there were many megalocytes in the blood and a typical megaloblastic marrow, in spleen puncture up to 13.6 per cent typical large megaloblasts were found (Fig 27, b). Mitoses were fairly frequent and the number of megaloblasts in the spleen was greater than that in the peripheral blood (1 megaloblast per 100 leucocytes) so that there can be no doubt



FIG 27 b c d Megaloblasts (14 per cent) in spleen puncture in pernicious anemia

- (b) May Grunwald stain a mitosis on the left
 (c) Round colourless gap in the otherwise basophil plasma of a young megaloblast (May Grunwald Giemsa)
 (d) Phase contrast picture Here an agglomeration of brightly shining globules (Kugelhaufen) can be seen (Zeiss phase contrast microscope 1000)

that these abnormal cells can arise in the spleen and it was striking that they were on the whole more mature in the spleen than in the sternal marrow. No obvious explanation is available but we have found a similar difference in the degree of maturation in chronic myeloid leukemia.

In early megaloblasts with the phase contrast microscopy (Moeschlin (23)) an agglomeration of brightly shining globules could be found (Kugelhaufen) which could not be observed in other cells. A round colourless gap in the otherwise basophil

plasma stained by May Grünwald Giemsa corresponds to these bodies which probably are a special form of mitochondria (see Fig 27 c)

TABLE 10b

Myelo-gram	Splenogram										
Megaloblasts	Plasmot retic cells	Pulp cells	Megaloblasts			Myelocytes		Neutrophils		Eosinophils	Lymphocytes
			Basophil	Polychrom	Oxyphil	Half mature	Mature	Stalk forms	Segmented		
57 0	2 4	0 6	2 4	10 6 0 6		2 2	3 6	4 6	11 0		
			13 6			5 8		15 6		3 4	53 8

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VII THROMBOCYTOPENIA WITH SPLENO-MEGALY

It is recognised that the spleen plays a considerable part in the genesis of many forms of thrombocytopenia (Naegeli, Rohr, Heilmeyer). At least two processes are at work viz inhibition of maturation of megakaryocytes in the marrow probably as the result

of a splenic hormone and also increased destruction of thrombocytes in the spleen itself. Any disease associated with splenomegaly may therefore, be accompanied by thrombocytopenia and if this is sufficiently severe with a *hæmorrhagic diathesis*. Thrombocytopenia of splenic origin has been observed in a very great variety of splenic diseases *e.g.* in such non infective enlargements as that of Gaucher's disease and splenomegalic cirrhosis and also in those of infective origin such as chronic malaria. In a woman aged 44 (No. 4 Table 10a) with a typical *hæmolytic anæmia* in this way we observed as a great exception an extreme and fatal thrombocytopenia (10 000 platelets per c mm.) although splenectomy was performed.

This case cannot be interpreted as an hormonal hypersplenism because the leucocytes were 7 800 per c mm. and the reticulocytes 16.6 per cent. It appears clear that in many such cases the splenic thrombocytopenia is mainly due to increased destruction of platelets by the spleen and that the increase of megakaryocytes in the marrow is a response to this increased utilisation of platelets.

In chronic Werlhof's disease in which splenomegaly although constant is never great the chief defect is still unknown but disturbance of the maturation of megakaryocytes appears to be one of the main factors. The pathological conditions found in the spleen vary from case to case (Heilmeyer-Schulten).

Then again in acute myeloblastic leukemia with splenomegaly there is often severe thrombocytopenia but here the principal factor is not to be found in the spleen but in the leukæmic proliferation in the marrow which leads to disappearance of megakaryocytes.

Spleen puncture is totally contra-indicated in a case of *hæmorrhagic diathesis* associated with severe thrombocytopenia. Diagnosis will have to depend upon careful analysis of the blood and sternal marrow. Only if the platelets temporarily increase and other methods reveal no tendency to *hæmorrhage* can spleen puncture safely be used. In the following case (Case 1) there was typical Werlhof's disease in a man of 35 in whom repeated blood transfusions succeeded in making splenectomy unnecessary. Spleen puncture showed an almost normal splenogram (Table 11) in which there was however some increase of eosinophiles and plasmacytoid reticulum cells. Platelets are as we have already mentioned more numerous in spleen punctures than in blood films. The presence of plentiful platelets in spleen puncture in Werlhof's disease obviously cannot be regarded as evidence of a thrombocytolytic activity of the spleen. In none of our 3 cases did we observe an increase of macrophages in fact they were rather fewer than in most other diseases (0.1-0.2 per cent). It seems very doubtful therefore whether the spleen in this case removed platelets from the blood by phagocytosis on the part of the reticulo-endothelial cells. When one remembers the extreme fragility of the platelets it would seem unlikely that they

would be removed in this manner and by analogy with red corpuscles by hemolysin solution in the spleen appears to be more probable (Gripwall Dameshek and Schwartz) probably in addition to hormonal inhibition of the marrow (Naegeli)

TABLE 11

Case	1	2	3
	Morbus Werthof	Toxi tuberc	Toxi allergic (nephrosclerosis)
Age sex	35 o	38 o	30 f
<i>Hemogram</i>			
Hemoglobin (°)	—	10—	53
Red corpuscles (in millions)	—	5 888	2 616
Thrombocytes	—	$\frac{1}{2}$ / — = 2 944	0 / —
Leucocytes	—	8 400	7 900
Myelocytes	—	—	1½
Neutrophiles	—	74½	73
Eosinophiles	—	6½	1½
Basophiles	—	1	½
Monocytes	—	10½	3
Lymphocytes	—	7½	20
Plasma cells	—	—	½
<i>Spleen gram</i>			
Macrophages	0.1	0.2	0.1
Plasma retic cells	0.8	1.0	0.1
Pulp cells	—	—	0.3
Erythroblasts	—	—	7.7
Myelocytes	—	—	0.2
Neutrophiles staff	1.7	3.9	1
Neutrophiles segmented	21.9	11.8	4.1
Eosinophiles	4.7	1.2	0.4
Basophiles	1.1	0.3	0.1
Monocytes	1.3	3.6	3.6
Lymphoblasts	—	—	0.5
Lymphocytes young	1.0	0.9	3.3
Lymphocytes old	67.4	77.0	75.9
Lymphocytes total	68.4	77.9	79.7
Plasma cells	—	0.1	1.5
Megakaryocytes	—	—	0.1

We have observed 2 cases in which thrombocytopenia was probably due to toxins and in which splenectomy was performed

Case 2 A man aged 38 had suffered from repeated bleeding for 2 years. The spleen was not enlarged the platelets were at first between 11 and 35 000 later 2-3 000 per a mm. the blood picture was otherwise normal. Sternal puncture showed great increase of megakaryocytes particularly of the immature forms but no other pathological changes. No method of treatment produced a rise of platelets and the spleen was therefore removed. Films made from it showed typical tuberculous epithelioid cells no myelocytes but fairly plentiful megakaryocytes (Table 11 Case 2). Histologically the spleen showed many calcified tubercles up to about 0.5 cm in diameter. The platelets did not rise after operation and death occurred a week later.

Autopsy revealed old calcified tuberculosis of the liver. The cause of death was pneumonia probably associated with extensive bronchiectasis.

In this case there was, almost certainly a toxic infective disturbance of the maturation of megakaryocytes due to tuberculosis of the spleen and liver and perhaps also to the chronic bronchiectasis. It was not therefore, an example of thrombocytopenia due to hypersplenism. Kellert, Zorini, Alessandri, Reggiani and Lapp have published similar cases of thrombocytopenic purpura associated with splenic tuberculosis.

The present observation shows that compensatory foci of megakaryocyte formation may develop in such cases a fact which had been noted previously by the pathologists Lang and Lubarsch. The following observation is of special interest.

Case 3. A woman aged 30 had an attack of polyarthritis in June 1934 and a month later presented signs of a severe hemorrhagic diathesis. In September she was admitted to the surgical clinic (Professor Clairmont). Red corpuscles 2 million, hemoglobin 33 per cent, platelets almost absent (about 2 000 per c mm), bleeding time more than 1 hour, coagulation time 2 minutes, reticulocytes 5.6 per cent, leucocytes 3 700 per c mm. The spleen was not palpable but was enlarged to percussion. Only blood was obtained when sternal puncture was performed. On the advice of Professor Naegeli, splenectomy was performed on September 14th. The histological diagnosis was that of a myeloid reaction associated with thrombocytopenia.

Following operation there was pyrexia and a relapse of the joint condition. There was no rise of platelets and a tentative diagnosis of *peliosis rheumatica* was made. For this reason large doses of pyrimidon were given. The temperature fell and the platelets slowly rose until they reached 120 800 per c mm on October 21st. In January 1936 the joints were again troublesome and the platelets had fallen to 17 000 per c mm. Mitral disease was now present (red corpuscles 2 800 000 per c mm, hemoglobin 74 per cent, leucocytes 6 600, the urine contained 0.15 per cent of albumin). A number of infected teeth were present. These were removed and pyrimidon was administered again, the platelets did not rise and death occurred on May 20th as a result of uremia.

The autopsy showed verrucose endocarditis of the mitral valve associated with considerable nephrosclerosis. The femoral marrow contained many megakaryocytes, some of which had greatly lobulated nuclei indicative of inhibition of the maturation.

This is an example of severe thrombocytopenia accompanying rheumatic infection and endocarditis with renal sclerosis. It seems probable that all these processes depend upon sensitisation to certain bacterial toxins. Unfortunately the condition of the marrow before death was not known, but the post mortem findings in the femur and the changes in the blood picture can be interpreted as indicative of interference with maturation of megakaryocytes due to allergic and toxic cause.

In *peliosis rheumatica* the platelets are not diminished (Heilmeyer) and can therefore be excluded as can also a hemorrhagic diathesis due to capillary damage. It is striking that splenectomy had no effect on the thrombocytopenia and that it was only when the temperature fell as a result of administering pyrimidon that there

was a rise of thrombocytes. It appears to us therefore that in this case the rheumatic process not hypersplenism was responsible for the thrombocytopenia. There are other similar cases to be found in the literature in which chronic joint rheumatism has been accompanied by splenomegaly and thrombocytopenic purpura (Veil).

Spleen films (Case 3 Table II) showed numerous megakaryocytes (about 1 per 1000 cells) together with greatly increased extra medullary myelopoiesis which perhaps because of the frequent and severe hæmorrhages was predominantly erythropoietic. This case is unique because even in chronic myeloid leukaemia we have

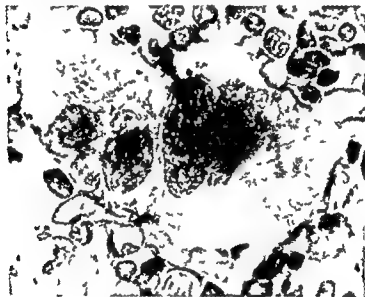


FIG. 8. Megakaryocytes in spleen films from a case of thrombocytopenic purpura.

never seen so many megakaryocytes (Fig. 28). Certainly in this case the condition was not leukæmic: the low percentage of myeloid cells (only 0.2 per cent. of myelocytes with increase of erythroblasts to 7.7 per cent.) and somewhat raised lymphocytes excluded this possibility while the excess of lymphatic plasma cells indicates the chronic inflammatory nature of the condition.

B. SPLENO-MEGALIES OF INFLAMMATORY ORIGIN

VIII. ACUTE AND SUBACUTE SPLENO-MEGALIES

Here are included those enlargements of the spleen that occur in some acute infections and which may pass over into a more chronic stage.

In this case there was almost certainly a toxic infective disturbance of the maturation of megakaryocytes due to tuberculosis of the spleen and liver and perhaps also to the chronic bronchiectasis. It was not therefore an example of thrombocytopenia due to hypersplenism. Kellert, Zorini, Alessandri, Reggiani and Lapp have published similar cases of thrombocytopenic purpura associated with splenic tuberculosis.

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Following operation there was pyrexia and a relapse of the joint condition; there was no rise of platelets and a tentative diagnosis of peliosis rheumatica was made. For this reason large doses of pyramidon were given. The temperature fell and the platelets slowly rose until they reached 120,800 per c mm on October 21st. In January 1936 the joints were again troublesome and the platelets had fallen to 17,000 per c mm. Mitral disease was now present (red corpuscles 2,800,000 per c mm, hemoglobin 74 per cent, leucocytes 6,600, the urine contained 15 per cent of albumin). A number of infected teeth were present. These were removed and pyramidon was administered again; the platelets did not rise and death occurred on May 20th as a result of uræmia.

The autopsy showed verrucose endocarditis of the mitral valve associated with considerable nephrosclerosis. The femoral marrow contained many megakaryocytes, some of which had greatly lobulated nuclei indicative of inhibition of the maturation.

This is an example of severe thrombocytopenia accompanying rheumatic infection and endocarditis with renal sclerosis. It seems probable that all these processes depend upon sensitisation to certain bacterial toxins. Unfortunately the condition of the marrow before death was not known, but the post mortem findings in the femur and the changes in the blood picture can be interpreted as indicative of interference with maturation of megakaryocytes due to allergic and toxic cause.

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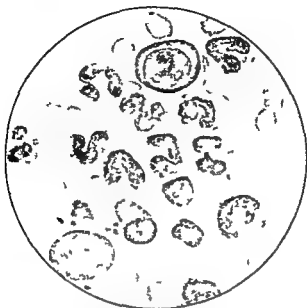


FIG. 11. Acute inflammatory spleen. Numerous toxic neutrophils, occasional myelocytes and macrophages and one young lymphatic plasma cell. (Painting by Mrs. Bollinger Schudel.)

The most striking feature (Table 12) was the very great increase of neutrophils (72 per cent) with only 19 per cent of lymphocytes with a marked shift to the left and toxic changes in the former. Plasmacytoid reticulum cells were also increased—an indication that the inflammation was of long duration while of course also

TABLE 12

Haemogram							Splenogram												
No of case	Leucocytes (in 1 000s)	Myelocytes	Neutrophiles staff	Neutrophiles segmented	Monocytes	Lymphocytes	Macrophages	Plasmacyt retic cells	Pulp cells	Myelocytes	Neutrophiles staff	Neutrophiles segmented	Eosinophiles	Monocytes	Lymphoblasts	Lymphocytes young	Lymphocytes old	Total lymphocytes	Plasma cells
1	73	4	76	15	1	7	0.3	3.2	0.7	1.7	63	192	0.2	1.2	0.1	19	172	192	12
2	74	—	39	44	9	6	0.6	0.4	0.5	0.7	<div>72.3</div> 24	<div>63</div> 49	0.8	8.2	—	54	737	289	0.5
											<div>59.5</div>								

The pathology and histology of acute splenic swelling (Lubarsch) is well known. We will only mention the congestion of the organ, the leucocytic infiltration of the pulp, the hyperplasia of the reticulo endothelium, sometimes accompanied by deposition of hæmosiderin and the occasional development of hæmopoietic foci (Hirschfeld).

The predominance of granulocytes in most acute infective diseases due to bacteria has recently been investigated (Ehrich *et al*). It is assumed that in virtue of the presence of their enzymes, corpuscular antigens are split into soluble antigens and so prepare for the anti body building cells (plasmacytoid reticulum cells). In this way the neutrophils contain mainly protein and carbohydrate splitting enzymes and the macrophages fat splitting ferments. This functional difference explains the conversion of the acute inflammatory picture (neutrophile reaction) into the chronic inflammatory one (monocytic plasma cell reaction).

Our observations have led us to divide acute inflammatory splenomegalies into two groups: those with a predominantly neutrophile response (bacterial infections) and those with a mainly lymphocytic one (virus infections).

1 NEUTROPHILE REACTIONS

The type occurs most commonly in acute septic diseases. We have not punctured the spleen in the early stages of such maladies because of the friability of the organ at this time. If however the disease persists and other methods have not made the diagnosis clear, spleen puncture can safely be performed if our technique is carefully followed, especially as a very thin needle can be used because of the cellularity of the pulp.

We have never punctured a typhoid spleen, but Vidal (1890) and Hayashihara (1909) often did so in order to obtain cultures and indeed the latter reported on 109 cases.

The main diagnostic value of spleen puncture in septic diseases is the demonstration of the causative bacteria. We have succeeded by this means after several blood cultures were negative. In other cases of pyrexia the explanation may be given by the finding of Dorothy Reed or tuberculous epithelioid cells (see pp. 104 and 117).

A woman of 36 had had high fever for 2 months with greatly increased erythrocyte sedimentation and leucocytes of 15-20 000 with much toxic change in the neutrophile granules. The spleen was just palpable. All cultural and agglutination tests were negative and after 6 weeks illness spleen puncture revealed the splenogram shown in Table 12 Case 1. No growth of pyococci was obtained and unfortunately, no attempts were made to grow tubercle bacilli but a few weeks later there was growth from a bilateral pleural effusion. The temperature fell after 2 months and recovery ensued from what must have been a severe hæmic spread of tuberculosis.

mononucleosis in an attempt to gain further information as to the origin of the characteristic cells. In 2 cases there was splenomegaly but not enlargement of lymph nodes while in another only the spleen and hilum glands were enlarged. As Chevallier has pointed out such purely splenomegalic cases are by no means uncommon. Occasionally the enlarged spleen may lead to so great a degree of inhibition of the marrow that the disease runs the course of agranulo-

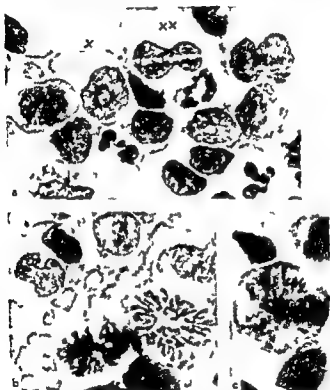


FIG. 30: Spleen puncture in infective mononucleosis

- (a) Numerous lymphoid monoblasts (x) and mature lymphoid monocytes (xx)—glandular fever cells
 (b) Mitoses in such cells
 (c) Abnormal (tripolar) mitosis in a young glandular fever cell

locytosis (Tidy and Heilmeyer (4)) we have seen one case in which the granulocytes numbered only 500 per mm. The rare thrombocytopenic cases (Bernstein Lloyd) are probably explicable in the same way.

In an earlier investigation (Moeschlin (5)) we found up to 11 per cent. of large reticulo endothelial cells in gland punctures at the height of the disease and among these there were many mitoses from which all transitions to the characteristic glandular fever

proving that spleen tissue not merely blood was present in the films. Myelocytes (17 per cent) were numerous and must have been derived from ectopic hemopoietic foci—a further sign of the chronicity of the inflammation. No epithelioid giant cells or any other indication of tuberculous infection could be found, so that it is clear that a severe hematogenous tuberculosis in its early stages produces changes in the spleen similar to those of any other septic infection.

The second example was a man of 63 who for 3 weeks, had suffered from cholangitis with great splenomegaly. The splenogram (Case 2 Table 12) showed marked neutrophilia associated with increase of monocytes (Fig. 32).

2 LYMPHATIC REACTIONS

Distinct splenomegaly can be detected in a number of diseases that are accompanied by a lymphatic response in the blood (lymphotropic virus diseases). These conditions, excluding lymphatic leukemia, which is not to be considered as being infective, will now be discussed in the light of spleen puncture.

(a) Infectious Mononucleosis

Nowadays this term is used to indicate a clinical picture which includes what used to be called lymphocytic angina, monocytic angina, lymphoid cell angina and Pfeiffer's glandular fever (see Lehndorff and Schwarz). The term mononucleosis is to be preferred to glandular fever because lymphadenopathy does not occur in all cases, especially in adults.

Heilmeyer regards all the cases as being of the same etiology, basing his view on the bacteriological and experimental work of Bland (1931), Wising (1939) and Berghe (1939). Schulten and Josephs, however, held that some diseases with a mononuclear blood picture are caused by other agents. Thus Josephs in America has described a condition resembling influenza in which extensive swelling of lymph nodes and a characteristic mononuclear blood picture occur during convalescence. Haberfeld also in South America has described a similar infectious condition which is conveyed by the bites of certain ticks. Hittman observed an epidemic of thrush with increase of lymphocytes including some atypical cells in the blood. We have reported lymphatic monocytoïd pictures in virus pneumonia (Moeschlin (10)), a few similar cells together with typical lymphatic plasma cells are fairly regularly seen in infective hepatitis and rubella. Lymphocytosis (up to 70–80 per cent) mainly due to increase of old lymphocytes is seen in lymphocytosis acuta (see p. 91). It seems probable therefore that further research, especially on viruses, may distinguish other groups.

We have punctured enlarged spleens in 8 cases of infectious

mononucleosis in an attempt to gain further information as to the origin of the characteristic cells. In 2 cases there was splenomegaly but not enlargement of lymph nodes while in another only the spleen and hilum glands were enlarged. As Chevallier has pointed out such purely splenomegalic cases are by no means uncommon. Occasionally the enlarged spleen may lead to so great a degree of inhibition of the marrow that the disease runs the course of agranu-

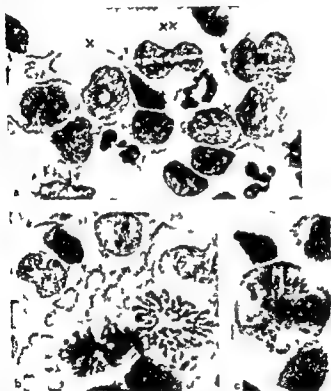


FIG. 30 Spleen puncture in infective mononucleosis

- (a) Numerous lymphoid monoblasts (x) and mature lymphoid monocytes (xx)—glandular fever cells
 (b) Mitoses in such cells
 (c) Abnormal (tripolar) mitosis in a young glandular fever cell

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In an earlier investigation (Moeschlin (5)) we found up to 11 per cent of large reticulo endothelial cells in gland punctures at the height of the disease and among these there were many mitoses from which all transitions to the characteristic glandular fever

cells ' could be discovered. We called these elements ' lymphatic monoblasts ' and the more mature cells arising from them ' lymphatic monocytes '.

First we will describe our observations on the structure of these reticulo endothelial cells as shown in our gland and spleen punctures , but as not all the cells present monocytoïd indentation of the nucleus and as the number of mature forms with lobed nuclei varies from case to case we shall here only distinguish ' lymphoid mono



FIG. 30 *d e f g* Phase contrast pictures

(d) Ordinary lymphocytes of blood. They show a fine greyish scanty granulation.

(e and f) Cells of infectious mononucleosis in blood. Contrary to the normal lymphocytes they show a relative coarse dark (black like) granulation which indicates a reticulo-endothelial origin. They are differentiated clearly from normal monocytes which contain no dark granulation. Fig. e shows a mitosis of an infectious mononucleosis cell in blood.

(g) A phase contrast photograph of two small lymphoid reticulum cells. They show in the plasma a dark granulation arranged in droplets which is similar to the inclusions of plasmacytoid reticulum cells and the cells of myeloma. These findings are perhaps in correlation with the production of certain globulins by these cells.

blasts and among the mature cells lobed and unlobed lymphoid monocytes.

The use of the word ' lymphoid ' rather than ' lymphatic ' is necessary because van Beck has shown that these cells are not formed only in the lymphatic system but also in the reticulo endothelium of the liver and perhaps of other organs. These elements are definitely pathological the formation being evoked by certain specific stimuli.

In the phase-contrast microscope these glandular fever cells show distinct coarse granules in the cytoplasm (Fig. 30 *e* and *f*) which are absent in the usual blood monocytes thereby and by the negative glycogen reaction (Gibb and Stowell) these cells differ also physically and chemically from the normal blood monocytes.

Structure of Lymphoid Monoblasts These are large rounded or oval cells (12-25 μ). The cytoplasm varies from pale to dark blue often being rather cloudy. Azure granules are absent from the youngest types but a few may be seen in the more mature cells. The nucleus which is round or oval occupies the greater part of the cell (14-20 μ) and may show early indentation. In the youngest forms the chromatin shows a fairly close but coarse meshwork (Fig. 30) in which lie 2 or 3 fairly large but rather indistinct nucleoli.

Methyl green pyronin stains the cytoplasm red (pale or deep) and the nucleus greenish blue. The peroxidase reaction is negative.

The coarseness of the nuclear texture and the histo-chemical characters clearly distinguish these cells from the oxydase positive myeloid monocytes.

Mitoses in monoblasts (Fig. 30 b) show so close a resemblance to those in plasmoblasts that they cannot be distinguished from one another in films although perhaps their chromosomes are a little shorter and broader. Occasionally atypical mitoses occur as in the case of the tripolar one shown in Fig. 30 c.

Lymphoid monoblasts can be distinguished from plasmoblasts by the closer and striate arrangement of the chromatin whereas that of the latter is quite delicate and evenly distributed. Some authors (Glanzmann, Leitner) refer to glandular fever cells as lymphoblasts but this is undoubtedly incorrect (Schwarz, Downey, Heilmeyer) because these are smaller, never have deep blue cytoplasm and exhibit more delicate nuclear structure.

Spleen punctures show that cytogenesis reaches its greatest intensity in the first week, perhaps persisting into the second week and then diminishing rapidly (Table 13). The changes in the spleen and lymph nodes commence before those in the blood and disappear earlier. At first mitoses in monoblasts are numerous. In Case 3 spleen puncture on the 15th day of the illness showed 0.8 per cent, i.e., mitoses in 13 per cent of the total monoblasts. In Cases 1 and 5 one third and a quarter respectively of the monoblasts were in mitosis.

The characteristic feature of the spleen and lymph nodes at the height of the reaction is the occurrence of many immature and mitotic reticulo endothelial cells (lymphoid monoblasts) and all transitions to the more mature elements seen in the blood can be detected.

The most immature elements are very scanty in the blood but even mitoses may be seen as we have observed in one case (also Schwarz, Nyfeldt, Angelini). The number of normal lymphocytes in the spleen and lymph nodes is somewhat decreased during the phase of reticulo endothelial irritation but during convalescence there is first increase of lymphocytes with broad cytoplasm and later of typical small ones.

At the height of the illness plasma cells (1-5 per cent) identical with those seen in rubella are regularly present in the blood but it is incorrect to follow Waitz who would refer to all cells with well marked cytoplasmic basophilia or with distinct chromatin

spokes as plasma cells. We assume that the morphological resemblances are due to the fact that both the lymphatic plasmoblast and the lymphoid monoblast have a common origin from the reticulo endothelium i.e. from the large lymphatic reticulum cell which forms the germinal centres in lymph nodes. Even so as described above differences are detectable in the very immature forms.

TABLE 13
Infective Mononucleosis

Case number	1	2	3	4	5	6	7
Day of disease	6th	10th	15th	23th	24th	?	28th
Wiganatzu-Ducher reaction	+	+	++	+++	+-	+-	2mal neg
<i>Hæmogram</i>							
Leucocytes	9 000	8 700	9 100	6 800	7 100	5 800	8 900
Neutrophiles staff	11½	15	14	2½	15	22	18½
Neutrophiles segmented	20	24	7	14	17	11	40½
Lymphocytes	6½	32	71	54	58½	43	29½
Plasma cells	1	1	3	½	—	½	—
<i>Splenogram</i>							
Macrophages	0.3	+	+	+	—	0.2	—
Plasmacyt retic. cells	11	20	11	0.2	18	28	0.6
Pulp cells	0.9	1.1	1.0	1.0	0.2	1.4	0.2
Erythroblasts	—	—	0.1	0.8	0.1	—	0.2
Myelocytes	0.3	0.8	0.1	0.2	—	0.1	0.1
Neutrophiles staff	8.4	4.8	7.4	7.6	3.0	12.1	5.3
Neutrophiles segmented	5.6	10.3	4.0	10.5	3.8	9.5	14.2
Eosinophiles mature	0.6	0.3	0.2	1.0	1.9	1.0	1.1
Basophiles mature	0.2	0.7	0.2	1.6	1.1	0.3	0.8
Monocytes	3.9	3.8	4.6	5.5	4.0	9.3	3.8
Lymphoblasts	—	0.1	—	—	—	—	—
Lymphocytes small young	3.0	5.8	2.5	4.5	5.8	4.5	2.8
Lymphocytes small old	59.3	4.2	42.4	29.4	62.7	41.1	65.0
Lymphocytes large young	0.9	1.6	0.1	0.1	1.1	0.8	0.8
Lymphocytes large old	3.3	15.4	5.9	9.5	4.5	5.3	2.8
Lymphocytes total	66.5	47.1	50.9	43.5	74.1	51.7	71.4
Lymphoid monoblasts (glandular fever cells)	1.2	2.6	4.5	0.2	1.6	0.4	0.2
Glandular fever cells unlobed	6.4	13	16.0	14.9	4.0	6.2	1.8
Glandular fever cells lobed	3.5	12.2	7.2	1.8	3.6	7.5	0.2
Total of lymphocytes and glandular fever cells	77.6	75.1	78.6	71.4	83.1	60.8	73.6
Plasmoblasts	—	0.1	0.1	—	0.1	—	—
Plasma cells half mature and mature	1.1	1.0	2.6	0.2	0.7	2.4	0.1

In spleen puncture at the height of the disease lymphatic plasma cells are only slightly increased (1.3 per cent) unlike the state of affairs in epidemic jaundice in which they are numerous.

As in all inflammatory conditions of the spleen myelocytes (0.1–0.8 per cent) and a few polychromatic erythroblasts can be found. Pulp cells are moderately increased in number (up to 1.5 per cent in Case 6) a sign of any inflammatory reaction. The scanty neutrophiles show a distinct shift to the left. Otherwise

apart from the occasional presence of tissue mast cells and isolated fat cells the splenogram presents no other peculiarities

Origin of Glandular Fever Cells in the Spleen Our earlier investigations on puncture of lymph nodes led us to accept the view that the essential feature was a reaction of the reticulo endothelial cells of the lymphatic follicles. It is probable that the majority of the lymphoid monoblasts in spleen films arise from the reticulo endothelium of the follicles whereby in lymphatic tissue they may arise from the 'large lymphatic reticulum cells

Van Beck and Haeck have shown clearly that the reticulo endothelial system of the liver also contributes to the glandular fever cells because in liver punctures they found great increase of reticulo endothelial elements with many mitoses. Heilmeyer, Rohr and Schleicher saw a similar reaction in the sternal marrow. It seems likely that Heilmeyer's case of mononucleosis accompanied by symptoms of nephritis and also the occurrence of mononuclear reactions in the cerebro spinal fluid (Huber Gsell) indicate that the reticulo endothelium of the kidneys and meninges are involved in some cases the latter also in the occasional encephalitic type (Geliebter).

All these observations force us to the conclusion that infective mononucleosis is not characterised by reaction purely of the lymphatic system but by one of numerous parts of the reticulo endothelium which are involved according to the localisation of the unknown cause. The disease therefore would have to be regarded as a secondary infective reactive reticulosis. It is thus possible that in the spleen it is not only the reticulo endothelial cells of the lymphatic follicles but also those of the red pulp that are involved.

This question cannot be decided on the basis of the older cases in the literature (Dubois Tremolieres Haken) but the more recent reports of 8 cases with spontaneous rupture in the spleen (Smith 1946 Vaughan 1946) seem to confirm the present view. Smith's case showed dense lymphoid infiltration of the spleen capsule and trabeculae. Darley found the red pulp completely infiltrated with lymphatic cells but he did not find any mitoses perhaps because the disease was past its maximum (17th day). In Ziegler's case there were lymphatic infiltrations in the liver kidneys spleen and lungs.

The only reports of spleen puncture in infective mononucleosis was made by Sigon and Weil. Sigon found a considerable increase of reticulo endothelial cells with many macrophages but we have not been able to confirm the latter finding. Weil unfortunately does not mention the period of illness when he performed his puncture but at a time when the blood picture contained 49 per cent lymphocytes and monocytoid cells the splenogram showed 28 per cent typical lymphocytes 19 per cent young lymphocytes and 43 per cent monocytes with 0.5 per cent mitoses.

Spleen puncture in infective mononucleosis is not really of

diagnostic value because a proper knowledge of the structural differences between leukemic cells and glandular fever (broad plasma ¹) should permit of differential diagnosis without difficulty. It is only in cases with very well marked monocytoid forms that confusion can occur and then sternal puncture would rapidly settle the question.

As a number of cases of spontaneous rupture of the spleen have now been reported we should strongly advise against spleen puncture in any case of infective mononucleosis in which the organ is tender.

(b) Epidemic Hepatitis

Epidemic hepatitis is another of the virus diseases in which distinct lymphatic reaction occurs. We are in a position to report on 3 cases in which the spleen was punctured in one of them twice (Table 14).

Between the 5th and 11th day of the illness the spleen showed an increase of large lymphatic plasma cells and of their precursors the half mature plasma cells and plasmoblasts (Fig 31 a) with many

TABLE 14
Hepatitis Epidemica

Case number	1	2	2	3
Day of disease	6th	9th	11th	14th
<i>Hemogram</i>				
Total leucocytes	8 600	11 900	5 300	15 700
Neutrophiles staff	18½	21	10	25
Neutrophiles segmented	15½	48	53½	33
Lymphocytes	37	16½	19	27
Plasma cells	17½	1	1	1½
<i>Splenogram</i>				
Macrophages	+	+	0.2	—
Plasmacyt retic cells	0.6	1.1	0.6	3.1
Pulp cells	0.2	0.2	2.7	1.4
Erythroblasts	—	—	—	0.1
Myelocytes	0.1	—	—	1.7
Neutrophiles staff	10.6	2.7	1.5	4.7
Neutrophiles segmented	7.3	6.1	8.3	6.9
Eosinophiles mature	2.4	2.6	2.9	2.9
Basophiles mature	0.9	0.3	0.3	0.5
Monocytes	7.3	1.5	3.4	2.3
Lymphoblasts	—	0.4	0.1	—
Lymphocytes small young	6.5	5.1	4.0	3.0
Lymphocytes small old	33.9	69.6	70.9	68.5
Lymphocytes large young	4.1	3.0	1.6	1.2
Lymphocytes large old	5.9	2.8	1.5	1.9
Lymphocytes total	50.4	80.9	78.1	74.6
Plasmoblasts	1.3	1.5	0.2	—
Plasmoblasts in mitosis	0.4	0.1	+	—
Plasma cells half mature	1.3	1.5	0.8	0.2
Plasma cells mature	17.6	1.6	1.0	0.6
Plasma cells total	20.2	4.6	2.0	0.8

mitoses. It would seem reasonable therefore to expect the greatest intensity of the reaction about the 3rd or 4th day of the illness because on the basis of our investigations of the blood and gland puncture picture in rubella we found that the maturation time of blood plasma cells is from 4 to 5 days (Moeschlin (2)). The fact that the number of neutrophils is so much lower than in the blood proves that the plasma cells are really derived from the spleen not from admixture of blood with the puncture fluid. Further the presence of mitoses and of very young forms also contradicts the idea that they may have been added into the spleen from the blood.

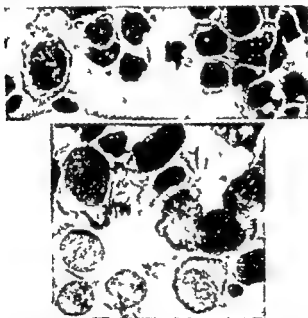


FIG. 31. Spleen puncture in infective hepatitis

- (a) Numerous lymphatic plasma blasts and young plasma cells
 (b) Mature plasma cells and large lymphatic elements some resembling glandular fever cells

The plasma cell reaction in the spleen diminishes fairly quickly as is shown in the spleen puncture of Cases 2 and 3. *The plasmacytoid reticulum cells are not increased in this stage of the disease—they do not appear to increase until the lymphatic reaction is beginning to subside (Table 14).* Also at the same time as the lymphatic plasma cells increase there is a tendency among the lymphocytes for the appearance of forms with wide cytoplasm and young nuclei just as in other types of lymphatic reaction (Fig. 31 b). These changes are morphologically more striking than is the exact percentage change of the individual cell types. Spleen puncture otherwise showed a

typical acute inflammatory reaction viz definite shift to the left of the rather diminished neutrophiles and the presence of a few myelocytes the latter mainly in the late stages in one case they reached 1.7 per cent. Erythroblasts were not seen. In the later stages, the pulp cells (Cases 2 and 3) as in the majority of inflammatory reactions of the spleen were fairly numerous.

The above findings thus show that during and probably even more preceding the presence of numerous large plasma cells in the peripheral blood there are active mitoses of such cells in the spleen from which all types of elements up to the most mature plasma cells pass into the blood. It is assumed that the majority of the plasma cells originate in the spleen because there is no similar reaction in the bone marrow (Landolt). It is in conformity with our findings in gland puncture in rubella that the similar cells in epidemic hepatitis are seen to originate from the lymphatic follicles of the spleen. Even in epidemic hepatitis a slight enlargement of cervical lymphatic glands may be detectable during the first 5 days although this is always less than in rubella or in infective mononucleosis presumably the difference depends upon involvement of the hepatolienal system in infective hepatitis and the lymphatic glands in rubella.

Plasmacytoid reticulum cells are constantly present in the spleen and it is tempting to suppose that young plasma cells arise from them and are really nothing more than specialised functional types. We do not share this view because in the early stage of infective hepatitis there is no increase of plasmacytoid reticulum cells in the spleen while Landolt found only normal numbers of these elements in the marrow (as we did also in rubella). He therefore agrees with our contention that the hæmic plasma cells cannot arise from plasmacytoid reticulum cells. Equally there is no evidence of transitions from young plasma cells (plasmoblasts) to plasmacytoid reticulum cells.

(c) Other Virus Infections Accompanied by Splenomegaly and Lymphatic Reaction

It has already been mentioned that several virus infections may be accompanied by a lymphatic reaction in which irritation cells may be found i.e. elements with wide basophilic cytoplasm or even with monocytoïd characters like those of glandular fever cells. Doubtless future research will enable the diseases due to lymphotropic viruses to be subdivided but at present this has only been done in the case of mononucleosis (Wising van Berghe) rubella (Steinmaurer) epidemic hepatitis (Siede Luz) and some of the virus pneumonias (Weir Horsfall).

(d) Q Fever (*Rickettsia Burneti*)

This disease described in Australia by Derrick (1937) runs an acute course and is accompanied by pulmonary infiltrations. Gsell

was the first to recognise the disease in Switzerland and it is now our impression that at least 60 per cent of cases of so called virus pneumonia or atypical pneumonia in Switzerland are due to such rickettsiasis

In some cases there is a lymphatic reaction and the spleen is constantly enlarged. Spleen puncture in a man of 24 on the 8th day of illness did not reveal any increase of lymphocytes (60 per cent) but many young basophilic types among which mitoses were numerous were seen while there was an associated increase of monocytes (13 per cent) neutrophils (19 per cent) and plasmacytoid reticulum cells (2 per cent). In the blood the lymphocytes reached their maximum (45 per cent of 7 100 leucocytes) on the 17th day

(e) Lymphocytosis Acuta

Smith (1941) described this disease which usually occurs in childhood. Gsell has seen some cases in Switzerland and we have observed two in adults in which the white cells rose to 15-20 000 (all small lymphocytes and no glandular fever cells) but fell to normal again in 2-3 weeks. Spleen puncture was not performed because neither lymphatic glands nor the spleen were enlarged but it is reasonable to assume that the number of lymphocytes would be lower than in leukaemic spleens. We doubt whether the case to be described in the next section is really an example of lymphocytosis acuta but the possibility cannot be excluded

(f) Non inflammatory Lymphatic Reactions (possibly of central origin)

We have seen one case of pituitary tumour in which there was a prolonged and progressive lymphocytosis so great that the condition was originally diagnosed as lymphatic leukaemia. Löffler in a personal communication has mentioned an example of extreme lymphocytosis in a similar case

In view of the rarity of such a condition one case in which the enlarged spleen was punctured will be described in more detail especially because 7 years observation have enabled lymphatic leukaemia to be excluded

S. H. aged 40 had suffered from headaches since a fall in February 1942 there was also diminished libido right sided exophthalmos and left abducens palsy. X ray of the skull in March 1942 revealed changes in the sella suggestive of pituitary tumour. Blood pictures —

April 17th 1942	leucocytes 4 800 with 59 per cent lymphocytes
June 24th 1942	leucocytes 4 300 with 56 per cent lymphocytes
July 21st 1942	leucocytes 4 200 with 46 per cent lymphocytes
August 19th 1942	leucocytes 11 200 with 79 per cent lymphocytes (14 380 per mm ³)

Admitted to hospital on August 25th 1942 with severe headaches and exophthalmos. Sella moderately enlarged. Spleen just palpable. No lymphadenopathy. Temperature never above normal. ESR 13 mm. haemoglobin 82 per cent. red corpuscles 4.1 million

leucocytes 19,800 reticulocytes 19 per cent platelets 39 440
 neutrophile staffs 5.5 per cent polymorphs 23.5 per cent eosino-
 philes 3 per cent monocytes 2.5 per cent and lymphocytes 65.5
 per cent (12 970 per cmm) The neutrophiles were structurally
 normal all the lymphocytes small and of old type no basophilic or
 large forms being found Sternal puncture unsatisfactory but mainly
 blood was obtained Spleen puncture see below

Later the white cells increased to a maximum of 23 700 with 1000
 lymphocytes After 2 weeks of arsenical treatment by mouth the leuco-
 cyte values became normal thus on September 25th 1942 the leucocytes
 were 4 800 with 34 per cent of lymphocytes As the diagnosis of pituitary
 neoplasm was uncertain radiotherapy was not tried and the patient was
 discharged

Re admission February 13th to September 18th 1943 Exophthalmos
 Tendon reflexes weaker on right bilateral decrease of temporal fields
 Sella unchanged Lumbar puncture nil abnormal Vision rapidly
 became worse Leucocytes 9 200 with 27 per cent of lymphocytes
 Repeated examinations failed to reveal any increase of lymphocytes
 Radiotherapy to pituitary was followed by gradual improvement of
 vision and decrease of headaches During an intercurrent attack of
 pneumonia the leucocytes rose to 16 700 with 5.5 per cent of lympho-
 cytes and this was not followed by a definite post infective lympho-
 cytosis (leucocytes 4 700 with 34 per cent lymphocytes)

No lymphocytosis was found at any time during the subsequent
 history of the patient but in July 1944 there was transient eosinophilia
 (27 per cent) In November 1944 the spleen was no longer palpable
 no enlarged glands could be found and the headaches had ceased while
 vision was restored to normal The white cells were 6 500 with 20 per
 cent lymphocytes In January 1945 there was a second attack of pneu-
 monia also not followed by lymphocytosis

In short then this was the case of a man of 40 who developed
 the typical signs of pituitary tumour and a gradually increasing
 lymphocytosis (up to 18 000 small mature lymphocytes) with slight
 splenomegaly The lymphocytosis disappeared after a course of
 arsenic and has not recurred during a 7 year period of observation—
 a fact which excludes the possibility that the whole picture could be
 due to leukaemic infiltration of the pituitary Then again the con-
 dition cannot have been a post infective lymphocytosis because its
 onset was so gradual and its intensity was so great and further
 no such phenomenon followed the two subsequent attacks of
 pneumonia Equally a diagnosis of infectious mononucleosis is
 not tenable on account of the complete predominance of typical
 small lymphocytes and their gradual increase over a period of
 months The idea of a very prolonged remission of chronic
 lymphatic leukemia was excluded by spleen puncture during the
 stage of lymphocytosis In that disease (see p 120) the lymphocytes
 in the spleen are increased to 92–99 per cent often with many
 immature and polymorphic cells whereas in the present case the
 splenogram was normal (Table 15) with 85 per cent of lymphocytes
 rather young but essentially normal cells with very few larger forms

Obviously in view of the transience of the lymphatic reaction it is possible that there was a fortuitous infection with lymphocytosis acuta in a person with a pituitary tumour. Löffler's observation (mentioned above) and the experimental work of Dougherty and White (1947) seem to us to suggest that a central (pituitary) influence has to be admitted as an explanation. It is possible that in association with other endocrine deficiencies there was a decrease of an adrenotropic hormone required by the adrenal cortex. The authors just mentioned found that increase of this hormone caused a fall in the number of lymphocytes while deficiency leads to increase of lymphocytes varying with the functional state of the adrenal cortex. Only further observations can settle the question.

TABLE 15

<i>Blood sm.</i>		<i>Erythroblasts</i>	0.9
Total leucocytes (in 1 000s)	0.7	<i>Myelocytes</i>	0.2
Neutrophiles staff	9	Neutrophiles staff	1.7
Neutrophiles segmented	18½	Neutrophiles segmented	6.0
Eosinophiles	2½	Eosinophiles	2.2
Basophiles	1	Basophiles	0.6
Monocytes	2½	Monocytes	1.6
Lymphocytes	66½	Lymphoblasts	0.4
		Lymphocytes small young	14.2
<i>Splenogram</i>		Lymphocytes small old	66.3
Macrophages	+	Lymphocytes large young	4
Plasmacyt retic cells	0.1	Lymphocytes large old	2.1
Pulp cells	0.7	Lymphocytes Total	81.4
Tissue mast cells	0.1	Plasma cells	0.5

IV. CHRONIC INFLAMMATORY SPLENOMEGALIES

The splenic enlargements belonging here may be primarily chronic as in sepsis lenta and Still's disease or may develop out of a preceding acute inflammatory splenomegaly (Fig. 32) such as that of malaria. Granulomatous conditions although really belonging among the chronic inflammatory states are considered in a separate section.

The pathological picture (Lubarsch) is characterised by degenerative changes such as deposition of ferruginous pigment and the occurrence of lipid cells. There are also exudative phenomena such as hyperæmia with leucocytosis, presence of plasma cells and sometimes of hæmopoietic foci with an associated hyperplasia of the reticulo endothelium. These changes give the splenogram its characteristic features.

Our observations have been on cases of sepsis lenta and of the rheumatic diseases viz Still's and Felty's diseases. The literature does not contain detailed descriptions of spleen smears in chronic inflammatory states except in malaria (Nagy 1924, Mele 1925).

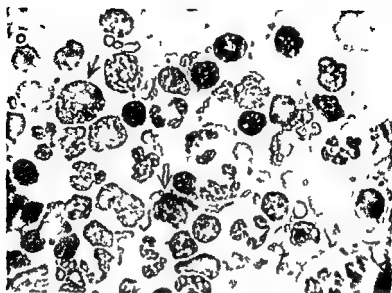


FIG. 33 Subacute inflammatory spleen with neutrophils monocytes occasional myelocytes erythroblasts and lymphopenia

Gosio 1944) and Kalishazar (Mele Nicolle etc) in which punctures were performed for demonstration of the causative parasites

Weil includes all chronic inflammatory splenograms in the group of macrophage reactions because he regards the presence of large numbers of macrophages as being the characteristic change

Characters of the Chronic Inflammatory Spleen film

The details vary from case to case and with the duration and intensity of the inflammatory process but they have certain features in common —

- (1) Increase of plasmacytoid reticulum cells and usually of large lymphatic plasma cells
- (2) Increase and shift to the left of the neutrophils often accompanied by increase of monocytes and lymphopenia
- (3) Variable number of erythroblasts and myelocytes
- (4) As compared with non inflammatory cases there is excess of pulp cells — usually of cell macrophages — and sometimes of hemosiderin macrophages

After a certain amount of practice a general examination of films enables one to recognise a chronic inflammatory spleen (Fig. 33 a) and in cases of diagnostic difficulty special attention is to be paid to the finding of the epithelioid cells typical of tuberculosis or the Dorothy Reed cells of Hodgkin's disease. The absence of the latter elements in a case of splenomegaly is strong evidence against a diagnosis of Hodgkin's disease because by the time the spleen is enlarged Dorothy Reed cells are usually plentiful in spleen

films. On the other hand failure to find epithelioid cells does not exclude tuberculosis because tubercles are irregularly scattered through the organ and may therefore escape puncture.

Some neoplasms: especially sarcomata accompanied by pyrexia with relative bradycardia and increased ESR may be accompanied by splenomegaly of chronic inflammatory type probably as a result of protein destruction.

In one case of sarcoma of the retroperitoneal muscles with fever about 100° spleen puncture showed 6.5 per cent plasmacytoid reticulum cells and 23 per cent neutrophils although at autopsy the distinctly enlarged spleen did not contain any metastases. Clinically the diagnosis lay between tuberculosis and Hodgekin's disease but the early onset of cachexia suggested neoplasia.

In another case with a chronic inflammatory type of splenogram and high fever caused by lympho sarcoma tumour cells were demonstrable in a second puncture of the spleen.

It is worth stressing the value of cultures from spleen punctures especially in those more chronic infections in which repeated blood cultures have been unsuccessful.

(a) Sepsis Lenta

In this condition the characters of the chronic inflammatory splenogram are particularly well marked (Table 16). Thus Case 4

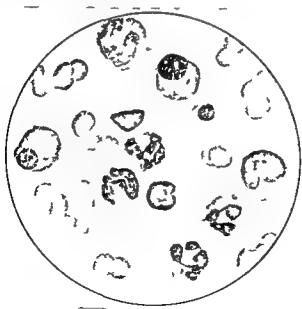


FIG 33 a

Chronic inflammatory spleen showing monocytosis, plasmacytoid reticulum cell, occasional erythroblasts, myelocytes and diminution of lymphocytes. (From the original drawing of Mrs. Bollinger Schudel.)

in which puncture was performed during a prolonged afebrile period showed 10.5 per cent of plasmacytoid reticulum cells (normal is 0.2-0.8) a figure only once exceeded in our experience in a case of hemolytic anemia associated with brucellosis and in one case of Still's disease.

In most cases the films contain an excess of pulp cells which are often seen as small syncytia of 2 or 3 cells (Fig. 12 c). On account of their irregular distribution the macrophages do not necessarily appear to be increased in the splenogram but in a general examination of films such elements containing cell debris are noticeable. In addition, hemosiderin macrophages may be numerous as may large monocytoid macrophages (Fig. 16 i k) sometimes (Case 2) with plentiful large lymphatic reticulum cells. In Case 4 we were also able to detect macrophages containing bacteria (Fig. 15 c). Neutrophils vary in number according to the stage of the inflammatory process but always show a shift to the left and sometimes distinct toxic granulation. Monocytes are considerably increased especially in the more prolonged cases but the percentage cannot be directly compared with that in the blood because lymphocytes are so numerous in the spleen. For this reason any figure above

TABLE 16
Sepsis Lenta

Case number	1	2	3	4
<i>Hemogram</i>				
Leucocytes (in 1 000s)	19	97	91	118
Myelocytes	—	1	—	—
Neutrophils staff	31½	25½	24½	20½
Neutrophils segmented	13½	40½	50	39
Eosinophiles	2	—	—	1
Basophiles	—	—	—	—
Monocytes	20	9	5	18½
Lymphocytes	33	24	18½	21
<i>Splenogram</i>				
Macrophages	0.8	0.3	0.1	0.1
Plasmaect retic cells	1.6	3.0	1.4	9.3
Pulp cells	6.8	1.5	0.3	2.5
Tissue mast cells	+	—	—	—
Fat cells	+	+	—	—
Erythroblasts	—	+	—	+
Myelocytes	—	1.4	—	0.1
Neutrophils staff	5.8	7.9	5.0	8.8
Neutrophils segmented	3.9	6.2	11.9	19.5
Eosinophiles mature	0.8	—	0.3	1.8
Basophiles mature	0.6	0.1	0.4	0.1
Monocytes	1.1	0.7	3.2	7.1
Lymphoblasts and large lymphatic reticulum cells	0.2	1.1	0.8	—
Lymphocytes small	72.2	63.3	63.2	46.7
Lymphocytes large	4.8	11.9	12.4	2.9
Lymphocytes total	77.0	76.5	76.4	49.6
Plasma cells	0.9	2.4	1.0	0.7

3 per cent is to be regarded as an excess. As a rule the lymphocytes tend to be rather younger and larger than normal even a few basophilic ones being present. Myelocytes and erythroblasts although less numerous than in acute cases are constantly found.

In one case we found many tissue mast cells (Fig 16 g h) and in two cases (Case 1 and 2) lipophages were numerous.

It is to be noted that films from chronic inflammatory splenomegaly (Fig 33 a) differ from normal in general appearance more than is shown by the numerical changes in the splenogram.

These findings in spleen films from Libman Sacks disease are the same as in sepsis lenta.

(b) Still Chauffard Felty Disease

This disease which belongs to the rheumatic group (Spuhler Zimmer) runs a chronic course accompanied by polyarthritis and splenomegaly which may become considerable. Sooner or later anemia, leucopenia and thrombocytopenia develop as a result of splenopathic inhibition of myeloid function while gradually progressive hæmochromatosis is also characteristic.

Splenectomy may cause improvement especially of the blood picture (Hatch, Büchler, Zimmer, Hirschboeck and our own observations).

In our own opinion there are no grounds for separating Still's disease from Felty's syndrome, the enlargement of lymphatic glands that occurs in the former condition but not in the latter depends mainly on the age of the patients. Some relationship certainly exists between these diseases and Libman Sacks disease and also cyclical agranulocytosis. In some typical cases of the Still

TABLE 17
Morbus Still Chauffard Felty

Case	1 44 ♂	2 35 ♀	Case	1 44 ♂	2 35 ♀
<i>Hæmogram</i>			<i>Pulp cells</i>	31	14
Total leucocytes	2400		Erythroblasts	02	+
Neutrophiles staff	45		Myelocytes	05	06
Neutrophile segmented	29		Neutrophiles total	21.7	16.6
Eosinophiles	3		Neutrophiles staff	12.0	13.0
Basophiles	11		Neutrophiles segmented	9.7	3.6
Monocytes	6		Eosinophiles	0.1	0.4
Lymphocytes	151		Basophiles	0.5	0.4
Plasma cells	—		Monocytes	2.0	2.0
<i>Myelogram</i>			Lymphoblasts	—	—
Plasmacyt retic. cells			Lymphocytes small young	5.1	2.4
(per 100 leucocytes)	14.4		Lymphocytes small old	57.6	58.6
Lymphoid retic. cells	28.8		Lymphocytes large young	0.7	0.4
<i>Splenogram</i>			Lymphocytes large old	1.5	2.8
Macrophages	0.6	1.2	Lymphocytes Total	59.9	64.2
Plasmacyt retic cells	10.3	11.4	Plasma cells	1.1	0.8

Chauffard Felty syndrome definite agranulocytic episodes occur periodically all 3 weeks (see next chapter)

We are in a position to report on 4 cases (1 man and 3 women) in all of which the spleen films were of the chronic inflammatory type with increase of plasmacytoid reticulum cells (up to 12.4 per cent in Case 2) and of monocytes. In one patient (Table 18 a) eosinophiles and lymphocytes were more numerous than in our other cases although many authors regard lymphatic hyperplasia sometimes with eosinophilia as characteristic of the disease.

Case 1 presents a number of important features and will therefore be recorded in some detail.

P. J. male aged 44

1935-36 in hospital for a long period suffering from rheumatic polyarthritis. 1936 polyserositis (?) rheumatic—guinea pig inoculation negative. 1937 further attack of pains in joints enlargement of glands spleen just palpable distinct leucopenia.

Diagnosis Still Chauffard Felty disease—gradual improvement but persistent pains and raised E.S.R. General condition poor even slight scratches fester—in November 1940 such an infection of a finger led to lymphangitis fistula in the axilla until patient admitted to hospital in 1943.

A thin man with atrophic skin no glandular enlargement and much pain in joints with slight swelling. Slight pyrexia. Spleen palpable (12 cm) and firm. Discharging sinus in right axilla—all examinations for T.B. negative.

Blood: red corpuscles 3.1 hæmoglobin 72 per cent reticulocytes 1.4 per cent leucocytes 2,400 lymphocytopenia of 15.5 per cent B.S.R. persistently high 68 mm Takata ++ Weltmann 0.35 serum protein 7.5 (upper limit of normal).

Sternal puncture great increase of plasmacytoid reticulum cells viz 14.4 per cent lymphoid reticulum cells also greatly increased—98.8 per 100 white cells. Slight shift to left of neutrophiles.

In hospital for 8 months but treatment had little effect. In 1945 he was admitted to another hospital in much the same condition no bone changes.

Spleen puncture revealed great increase of plasmacytoid reticulum cells (10.3 per cent) as shown in Table 17 (Case 1). In addition to the typical appearances of a chronic inflammatory splenomegaly there were slight lymphocytopenia moderate monocytosis increase of neutrophiles and distinct increase of macrophages. In distinction from the sternal marrow there was no increase of other reticulum cells which in the marrow was presumably attributable to a reactive proliferation resulting from chronic inflammation (see later in the discussion of reticulosis).

The remarkable increase of plasmacytoid reticulum cells in the spleen and marrow was associated with a much increased B.S.R. (68-72 mm) and a persistently positive Takata reaction. Even so myeloma could be excluded because of the clinical course and the normal characters of the plasmacytoid reticulum cells.

Our view is that the blood changes are related to increase of certain globulin fractions which are doubtless formed at least to a considerable extent by the plasmacytoid reticulum cells of the marrow and spleen. Perhaps the small lymphoid reticulum cells also play a part in the increased formation of globulins although these elements do not seem to increase primarily but only reactively. Waldenström's (1948) observations on diffuse proliferation of reticulum cells in the marrow associated with the presence of globulin of high molecular weight in the blood are probably significant in this connection. We have to ask ourselves if this case does perhaps belong to the above group as well but unfortunately it cannot be decided yet.

(c) Cyclical Agranulocytosis in the Felty Syndrome

This is a remarkable condition in which at fairly regular intervals of 3 weeks there are febrile attacks accompanied by neutropenia and such episodes may continue for years. Two forms of the malady

TABLE 18a
Cyclical Agranulocytosis (Male aged 20)

Date	5/42	1/44	1/45	Date	5/4	12/44	1/45
	Puncture	Film			Puncture	Film	
<i>Hæmogram</i>				Erythroblasts	—	—	—
Total leucocytes				Myelocytes	—	—	—
(in 1 000s)	5.1	4.8	3.4	Neutrophiles staff	2.9	0.1	0.3
Neutrophiles staff	12½	1	3	Neutrophiles segmented	3.2	0.1	0.6
Neutrophiles segmtd	9	1	7	Eosinophiles	4.3	4.1	2.2
Eosinophiles	10	18½	11	Basophiles	0.3	0.4	0.7
Basophiles	1	—	2	Monocytes	8.3	3.9	6.4
Monocytes	27	23	24	Lymphoblasts	0.3	—	0.1
Lymphocytes	41	56½	53	Lymphocytes small young	7.6	4.8	10.9
Plasma cells	—	—	—	Lymphocytes small old	69.6	81.0	71.4
				Lymphocytes large young	1.9	1.5	1.6
<i>Splenogram</i>				Lymphocytes large old	1.7	2.9	4.6
Macrophages	1	0.2	0.1	Lymphocytes Total	80.6	90.2	88.6
Plasmacyt retic cells	0.2	0.3	0.4	Plasma cells	0.2	0.5	0.5
Pulp cells	—	0.2	0.2	Eosinophiles per 100 gran	22½	4.9	0½

have to be distinguished viz. one with periodic oral ulcers but no polyarthritides and an arthritic type.

We have records of a man now aged 26 in whom these phenomena have been observed for 6 years. It was a typical case of the Still-Chauffard-Felty syndrome with a large spleen, chronic polyarthritides and gradually progressive hemochromatosis associated with rheumatic endocarditis of the mitral valve. Every 3 weeks there was an attack of neutropenia with increase of monocytes and aggravation of the arthritides. During such phases sternal puncture showed distinct immaturity with disappearance of the more mature granulocytes.

This case has been published in detail by Löffler and Maier and a very similar one has since been recorded by Muller and Meyrieux (1948). Then as already mentioned there are cases with no rheumatic manifestations but with oral ulcers in the agranulocytic stage (Imerslund 1942, Vahlquist 1946, Reimann 1948, Barling 1948). In the latter type of case the spleen does not seem to be enlarged. It seems probable that the cases with polyarthritis are nothing more than cyclical agranulocytosis complicated by rheumatism.

Spleen punctures which were performed during apyrexial freedom from symptoms revealed distinct increase of eosinophiles on both occasions viz. 4.3 and 4.1 per cent (normal is 0.2-1.5 per cent). If the number of eosinophiles is calculated relative to the total granulocytes it will be found that on the first occasion there were 40 per cent and on the second 21 per cent. Such eosinophilia could also be confirmed in 1945 (2.2 per cent i.e. 18 eosinophiles per 100 granulocytes) and by histological examination of the spleen. Apart from distinct increase of monocytes and lymphocytes puncture fails to reveal other changes and there was certainly no increase of plasmacytoid reticulum cells.

The splenic and hemic eosinophilia in the absence of any evidence of parasite infestation strongly suggested an allergic basis for the disease.

(d) Malaria

Owing to the rarity of malaria in Switzerland we have records of only two punctures. Case 1 was a severe chronic tertian infection with great splenomegaly in which no treatment had been given. Case 2 was that of a man who had been infected with tertian malaria about a year earlier but who in spite of freedom from relapses still had a large hard spleen.

In both cases we found macrophages containing melanin and plasmodia as described by Melé (1925) and Gosio (1944). As long ago as 1924 Nagy pointed out that the parasites might easily be found by spleen puncture even in cases in which repeated examination of the blood had failed to reveal them. And as Gosio stresses the finding of the typical malaria pigment is of special importance in diagnosis. This is dead black unlike the greenish yellow of hæmosiderin which is of course increased in amount in all chronic hæmolytic conditions. Fig. 33 b & c shows such malaria macrophages (from Case 2) filled with large aggregations of black pigment (This should be compared with the larger pigmented cells of the malignant melanoma (Fig. 53 f & g) which have a much broader cytoplasm). Malarial pigment may also be found in larger and smaller cells as well as ingested by granulocytes (Gosio) while it and hæmosiderin may also be seen lying free in films. *The presence of melanin*

in spleen puncture can confirm a diagnosis of malarial splenomegaly even when no parasites can be found



FIG 33 b c Malaria melanin

Macrophages contain melanin. Dark black rounded masses of pigment are characteristic and can easily be distinguished from the yellowish green or brownish yellow tint of hemosiderin (spleen puncture)

Demonstration of the Parasites Some of the parasites lie inside red corpuscles while others lie in a rosette arrangement round the nuclei of macrophages. Gosio stresses the fact that no parasite may be discoverable in the spleen between attacks although he did find quartan ones even after months of freedom from recurrence. During attacks he observed macrophages that had ingested red corpuscles which themselves contained parasites.

Spleen punctures regularly show an increase of plasmacytoid reticulum cells, some erythroblasts and myelocytes with excess of granulocytes and monocytes, a typical chronic inflammatory splinogram.

TABLE 18b

Malaria

Case	Macrophages Melanin macrophages Plasmacyt retic cells	Pulp cells	Metamyelocytes	Neutrophils		Eosinophiles	Monocytes	Lymphocytes	
				Stab	Segmented			Young	Old
1 55 ♀	- + 30 (30)	16	18	32.0	30.4	-	1.2	0.2	29.8
				62.4				30.0	
2 33 ♂	3.5 + + + (3.5)	3.0	-	14.0	16.0	0.5	2.0	2.0	59.0
				30.0				61.0	

(e) Kala azar (*Leishmaniasis interna*)

Until recently most spleen punctures have been performed for the demonstration of *Leishmania donovani*. After a prolonged period of septic pyrexia leukopenia and anemia and sometimes enlargement of lymphatic glands this disease which is indigenous to the Mediterranean countries South America and Asia leads to great splenomegaly (Nicolle Benhamou etc.)

The parasites both lying free and in macrophages are numerous in spleen films. Weil describes them as follows —

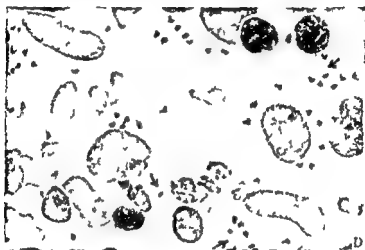


FIG. 33 d. Kala-azar

Typical leishmanias can be seen in a group of macrophages in a typical chronic inflammatory spleen puncture. A small punctate centrosome can also be clearly seen () in every leishmania.

In spleen films the causative organisms (2-4 μ) are oval rounded or pyriform. These organisms which are always numerous are mainly intracellular although some are seen scattered in small groups which somewhat resemble masses of platelets. Careful observation however reveals distinct differences. Thus they are each surrounded by a delicate transparent membrane which encloses pale blue cytoplasm which contains 2 small differentiated structures. The larger rounder one which lies eccentrically is the violet nucleus while the smaller basophilic one usually staff shaped is the centrosome.

We owe the films from which the following splenograms were made to the kindness of Dr Ferreras of Barcelona.

The changes are those characteristic of a chronic inflammatory spleen although lymphocytes are rather more numerous than usual over shadowing the monocytes and neutrophils.

In Case 1 there is great increase of plasmacytoid reticulum cells which seems to be characteristic of the disease both in the spleen and the sternal marrow (Leitner). This is accompanied by an

TABLE 18c

Kala a ar

Case	Macrophages Leishmanin-macrophages Plasmacytic cells	Pulp cells	Basophilic Polychromatic Erythroblasts	Myelocytes Half mature Mature Meta	Neutrophils Stab Segmented	Eosinophiles	Basophiles	Monocytes	Young Lymphocytes Old	Lymphatic plasma cells
1	05 13 68 86	—	— 05 17 17	— 03 70 3	47 20 67	—	—	08	37 75 6 79 3	05
2	08 + 10 18	06	02 04 12 18	06 — 32 38	100 10 110	02	02	— 6	24 75 6 78 0	—

increased B S R The macrophages were mainly seen in groups in the films

Gatto also saw increase of plasmacytoid reticulum cells and a slight extramedullary myelopoiesis (28 cases)

The occurrence of splenopathic inhibition of the marrow in chronic cases with great splenomegaly is an occurrence to be borne in mind (Cartwright 1948)

(f) Histoplasmosis

We have not had the opportunity of examining a case but presume that the parasites would be found inside reticulum cells in the spleen The organisms show 2 or 3 large rounded structures resembling a saucer 1 to 10 being found in each affected macrophage (see Wintrobe Plate XIII) but as far as we are aware they have up to the present time only been demonstrated histologically

X GRANULOMATOUS CONDITIONS

Diseases in which the causative organisms evoke the formation of a specific type of granulation tissue belong to this group We shall however only discuss tuberculosis sarcoidosis syphilis and undulant fever leaving Hodgkin's disease the nature of which infective or neoplastic to a separate section

(a) Tuberculosis

A definite diagnosis of tuberculous infection can be made by spleen puncture only if elements characteristic of the disease are

found *vi.* epithelioid or Langhans giant cells or if the bacteria can be demonstrated by staining or culture. Our own observations on gland puncture as well as those of Stahel and Tischendorf indicate that the characteristic changes do not develop in less than 2 or 3 weeks after infection.

We have records of 5 cases in which the specific elements were found in spleen films. Nassau (1926) recorded a case of sepsis with splenomegaly in a child in which a diagnosis of miliary tuberculosis was made by finding tubercle bacilli in films from spleen puncture. Weil (1936) aspirated caseous material in which no tubercle bacilli could be found although it was capable of infecting a guinea pig.

Epithelioid and giant cells have been found in gland puncture by Klima and Fleischhacker (1937), Tischendorf (1939), Stahel (1939), Leitner (1940) and Forteza (1947) but there are no previous records of their being found in spleen punctures.

Structure of Epithelioid and Langhans' Giant cells

Epithelioid cells (20–35 μ) are usually seen as elongated oval or slightly pointed elements often lying in small groups. The oval pale violet reddish or deep violet nucleus (14–20 μ) consists of a characteristic arrangement of narrow rods of chromatin with intervening spaces such that one has the impression of looking through a fine network (Fig. 34 *a*). In the younger forms a small pale blue or violet nucleolus may be seen but this is not visible in the older cells which may indeed show some degree of pyknosis leading to a coarser arrangement of the chromatin (Figs. 34 *b* and *c*).

The pale blue or greyish cytoplasmic ring is relatively wide in young cells and becomes paler and more violet in older ones (Fig. 35) which may contain vacuoles and fine violet granules. The edge of the cell body is rather indistinct so that when these elements lie in groups it may not be possible to separate them.

The multi-nucleated Langhans giant cells present the same kind of nuclear structure as that of epithelioid cells but more often with some pyknosis. Stahel believed that these elements as seen in gland puncture arise from fusion of the epithelioid cells. In our opinion these cells probably arise from toxic inhibition of mitosis the nuclei undergoing division without division of the cytoplasm (polyploid cells) as in the case of Sternberg cells of Hodgkin's disease and multinucleated tumour cells (see also p. 43). Thus it is possible to find every transition between cells with 2 or 3 nuclei and enormous elements (70–90 μ) with the characteristic palisade arrangement of their numerous nuclei. In the cell shown in Fig. 36 it is possible to distinguish 41 nuclei but the typical arrangement found in sections may be disturbed in the making of films.

Both epithelioid and Langhans cells can be distinguished from Dorothy Reed cells because the nuclei of the latter are larger and rounder while the chromatin has a more granular arrangement.

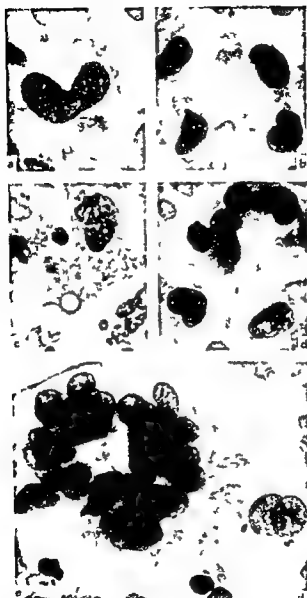


FIG. 34. Tuberculous epithelioid cells from tuberculosis of the spleen

- (a) Typical young type
- (b) Older form
- (c) Disintegrating cell
- (d) Fusion of several epithelioid cells to form a multi-nucleated element
- (e) Langhans' giant-cell from chronic tuberculosis of the spleen

with one or more distinct dark blue violet nucleoli while the cytoplasm is blue as compared with the pale greyish violet of that of epithelioid cells (Plate II Fig 37)

We can report on the *splenograms* of 7 cases (Table 19) which can be classified as follows —

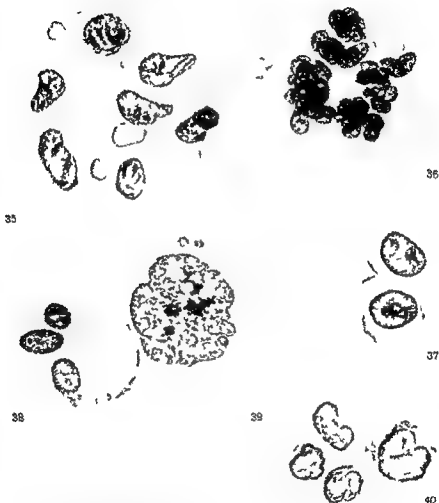
- (1) Acute hematogenous tuberculosis
- (2) Miliary tuberculosis
- (3) Chronic tuberculosis of the spleen

(1) *Acute Hematogenous Tuberculosis* In one severe case with prolonged fever leucocytosis and splenomegaly in which tubercle bacilli were grown from a pleural effusion the splenogram was characteristic of an acute inflammatory reaction with neutrophilia shift to the left and lymphocytopenia (see pp 80 and 81, Table 12, Case 1) There is in fact no difference between such splenogram and that of any other acute inflammatory one Stahel and Tischendorf found a similar state of affairs in the early stage of glandular tuberculosis viz a predominantly leucocytic response

TABLE 19
Cases of Tuberculosis

Case	1	2	3	4	5
Age Sex	30 +	38 o	61 ♀	18 o	28 o
<i>Hemogram</i>					
Total leucocytes	3 400	—	12 600	—	—
Neutrophiles staff	75½	—	18½	—	—
Neutrophiles segmented	—	—	68½	—	—
Eosinophiles	4½	—	—	—	—
Basophiles	—	—	—	—	—
Monocytes	8½	—	10½	—	—
Lymphocytes	11½	—	2	—	—
Plasma cells	—	—	—	—	—
<i>Splenogram</i>					
Macrophages	0.4	0.2	0.1	—	0.4
Plasmacyt retic cells	1.4	1.0	3.1	0.1	0.2
Pulp cells	1.5	—	1.0	1.1	1.2
Erythroblasts	0.2	—	—	—	—
Myelocytes	0.5	—	0.5	—	0.2
Neutrophiles staff	17.7	3.9	2.6	14.1	5.6
Neutrophiles segmented	9.4	11.8	11.9	14.6	5.4
Eosinophiles	2.5	1.2	—	1.5	1.6
Basophiles	0.1	0.3	0.1	0.4	0.6
Monocytes	20.3	3.6	3.4	7.4	2.2
Lymphoblasts	—	—	2.8	—	—
Lymphocytes small young	0.7	0.3	2.8	3.1	2.4
Lymphocytes small old	41.7	75.8	71.3	53.8	75.0
Lymphocytes large young	0.2	0.6	0.6	1.4	0.4
Lymphocytes large old	0.7	1.4	2.4	1.5	1.2
Lymphocytes Total	43.3	77.9	77.1	59.8	80.0
Plasma cells	0.3	0.1	0.2	0.6	0.4
Epithelioid cells	2.3	—	—	0.4	1.4
Langehans giant-cells	0.1	—	—	—	—

PLATE II PATHOLOGICAL FORMS OF RETICULO-ENDOTHELIAL CELLS IN
SPLEEN FILMS (From paintings by Mrs Bollinger Schudel)



- FIG 35 Epithelioid cells of tuberculosis
 FIG 36 Langhans giant-cell
 FIG 37 Young Dorothy Reed (Sternberg) cell with typical blue nucleoli
 (Hodgkin's disease)
 FIGS 38 39 Multi-nucleated giant-cell Dorothy Reed cells
 FIG 40 Pathological reticulum cells (neoplastic) from a case of primary
 malignant reticulosis (reticulo-endotheliosis)

with one or more distinct dark blue violet nucleoli while the cytoplasm is blue as compared with the pale greyish violet of that of epithelioid cells (Plate II Fig 37)

We can report on the *splenograms* of 7 cases (Table 19) which can be classified as follows —

- (1) Acute hematogenous tuberculosis
- (2) Miliary tuberculosis
- (3) Chronic tuberculosis of the spleen

(1) *Acute Hematogenous Tuberculosis* : In one severe case with prolonged fever leucocytosis and splenomegaly in which tubercle bacilli were grown from a pleural effusion the splenogram was characteristic of an acute inflammatory reaction with neutrophilia shift to the left and lymphocytopenia (see pp 80 and 81 Table 12 Case 1) There is in fact no difference between such splenogram and that of any other acute inflammatory one Stahel and Tischendorf found a similar state of affairs in the early stage of glandular tuberculosis viz a predominantly leucocytic response

TABLE 19
Cases of Tuberculosis

Case	1	2	3	4	5
Age sex	30 ♀	38 ♂	61 ♀	18 ♂	78 ♂
<i>Hemoqram</i>					
Total leucocytes	3 400	—	12 600	—	—
Neutrophiles staff	75½	—	18½	—	—
Neutrophiles segmented	—	—	68½	—	—
Eosinophiles	4½	—	—	—	—
Basophiles	—	—	—	—	—
Monocytes	8½	—	10½	—	—
Lymphocytes	11½	—	2	—	—
Plasma cells	—	—	—	—	—
<i>Spl noqram</i>					
Macrophages	0.4	0.2	0.1	—	0.4
Plasmacyt retic cells	1.4	1.0	3.1	0.1	0.7
Pulp cells	1.5	—	1.0	1.1	—
Erythroblasts	0.2	—	—	—	—
Myelocytes	0.5	—	0.5	—	0.2
Neutrophiles staff	17.7	3.9	2.6	14.1	5.6
Neutrophiles segmented	9.4	11.8	11.9	14.6	5.4
Eosinophiles	2.5	1.2	—	1.5	1.6
Basophiles	0.1	0.3	0.1	0.4	0.6
Monocytes	20.3	3.6	3.4	7.4	2.7
Lymphoblasts	—	—	—	—	—
Lymphocytes small young	0.7	0.3	7.8	3.1	2.4
Lymphocytes small old	41.7	75.8	71.3	53.8	75.0
Lymphocytes large young	0.2	0.6	0.6	1.4	0.4
Lymphocytes large old	0.7	1.2	2.4	1.5	2.2
Lymphocytes Total	43.3	77.9	77.1	59.8	80.0
Plasma cells	0.3	0.1	0.2	0.6	0.3
Epithelioid cells	2.3	+	+	0.4	1.4
Langhans giant-cells	0.1	—	—	—	—

(2) *Miliary Tuberculosis* The splenogram of Case 3 was from a woman of 61 with chronic pulmonary tuberculosis who suddenly developed pyrexia and a palpable enlargement of the spleen. A suspicion of miliary tuberculosis was naturally aroused but the diagnosis was not definitely confirmed by radiography.

Spleen films were typical of a chronic inflammatory condition viz increase of plasmacytoid reticulum cells (31 per cent) pulp cells myelocytes (0.5 per cent) and slight monocytosis without increase of neutrophils. At one place in the film 2 typical epithelioid cells were seen (Fig. 34 a). The further course of the illness and the autopsy at which recent tubercles were found in the spleen confirmed the diagnosis.

(3) *Chronic Tuberculosis of the Spleen* Some details of Case 1 which was of exceptional interest will be given.

G. K. aged 30 saleswoman. Not well since pleurisy with effusion at age of 30. Irregular fever up to 101° F. heaviness in upper abdomen and enlargement of the spleen.

In hospital the temperature varied up to 102° F. sweating spleen greatly enlarged reaching iliac crest. Liver enlarged (15 cm). Liver function tests were within normal limits. Mantoux 1:100 000 negative 1:10 000 positive. W.R. negative. Brucella test negative. B.S.R. 43 mm.

Blood Haemoglobin 76 per cent reds 3.5 million reticulocytes 1.6 per cent constant leukopenia 3 400 neutrophils 77 per cent eosinophiles 3.5 per cent (sometimes up to 8 per cent) monocytes 6 per cent lymphocytes 13.5 per cent platelets 76 000.

Spleen Puncture 156 nucleated red cells per 100 white cells slight immaturity of granulopoiesis and some increase of metakaryocytes.

Hodgkin's disease was suspected but radiotherapy neither improved the general condition nor reduced the size of the spleen.

Spleen Puncture Typical epithelioid cells found and tuberculous infection was also confirmed in an excised cervical lymphatic gland.

Splenectomy was performed in the Surgical Clinic (Prof. Clairmont) 28 cm long and 1 740 g in weight. *Histologically* (Pathological Institute Zurich Prof. von Meyenburg) the serous coat was smooth but greyish nodules could be seen through it. On section there were many subcapsular greyish nodules which in some areas were in groups. Microscopically these were composed of epithelioid and Langhans giant cells only remnants of pulp with dilated sinuses lay between the nodules. The pulp cords were infiltrated with plasma cells and to a less extent with eosinophiles and neutrophils. No follicles could be recognised.

Thus this is a case of isolated nodular tuberculosis of the extremely enlarged spleen (28 cm and 1 740 g) with a long period of intermittent fever. Spleen puncture revealed the presence of epithelioid cells and a splenogram characteristic of a chronic inflammatory reaction with an unusual degree of monocytosis (20 per cent instead of the normal maximum of 2.5 per cent). Another case has already been discussed in the section on thrombocytopenic

culosis splenectomy with prophylactic streptomycin treatment is the therapeutic of choice

b Sarcoidosis (Boeck)

In about one third of the cases of pulmonary sarcoidosis the spleen is also involved (Löffler and Dressler Löffler and Jaccard) but the spleen may be the only affected organ (Secretan Gebattel Askanazy Friedmann)

We have no experience of spleen puncture in a proven case of

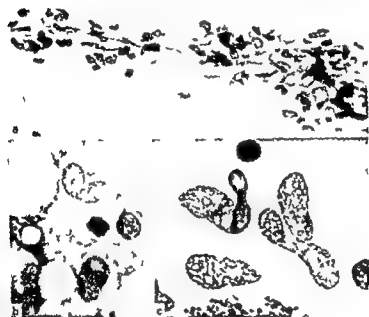


FIG. 41 a b c Epithelioid cells

(a and b) *Sarcoidosis* (Boeck) general and detail views. Delicate network of chromatin and distinct nuclei and arrangement of the cells in draught of fish (gland puncture)

(c) *Alveolar* (Bang) Note the large size of the cells and the relatively large masses of chromatin (spleen puncture)

sarcoidosis and are not aware of any reports in the relevant literature. In one doubtful case (Case 5 Table 19) with hilar lymphomata spleen puncture revealed many young epithelioid cells but as the Mantoux test was positive (1:100 000) a definite diagnosis could not be made.

Histologically, sarcoid granulomata in the spleen and other organs closely resemble tuberculosis (Löffler Dressler) but generally without any sign of caseation.

In one patient with hilar lymphomata we found the characteristic epithelioid cells in groups and garlands by puncture of

splenomegaly (p 77) Case 4 which we owe to the kindness of Professor Rohr showed numerous epithelioid cells in its chronic inflammatory type of splenogram

Summary In recent miliary spread spleen films are of acute or subacute inflammatory type viz increase of neutrophiles with leftward shift some myelocytes and perhaps monocytosis and lymphocytopenia At this stage diagnosis has to depend upon the finding of acid fast bacilli because the characteristic granulation tissue has not yet been formed

In more prolonged cases of hemic spread spleen puncture shows ordinary chronic inflammatory changes with increase of plasmacytoid reticulum cells and perhaps, neutrophilia with shift to the left monocytosis and lymphocytopenia Of course if the needle penetrates the specific granulation tissue there will be many epithelioid cells and sometimes giant cells Such appearances with striking monocytosis were found in Case 1 already described

Thrombocytopenic purpura probably of mixed toxic infective and hormonal origin (inhibition of maturation of megakaryocytes by hypersplenism) is by no means uncommon in association with tuberculosis of the spleen (see Case 2 Table II and also reports of Kellert Zorini Alessandri Weiner Reggiani and Lapp)

Diagnostic Value Spleen puncture is in our opinion of special value in cases of tuberculosis confined to the spleen because by examination of several films (and repeated puncture if necessary) it will usually be possible to demonstrate epithelioid cells But even in miliary cases which often cause diagnostic difficulties spleen puncture may be useful always remembering to stain at least one film for acid fast bacilli which in Nassau's case made a difficult diagnosis clear It is of course also valuable to use some of the puncture fluid for culture and animal inoculation

In cases of persistent pyrexia with splenomegaly in which malaria sepsis lenta chronic cholangitis and undulant fever can be excluded failure to find signs of specific granulation tissue in an otherwise typical chronic inflammatory splenogram is in favour of tuberculosis rather than of Hodgkin's disease which is usually so diffuse as to be demonstrable in repeated puncture If cachexia be pronounced with fever increased B S R and perhaps a chronic inflammatory splenogram the possibility of sarcoma will occur to the mind (see p 95)

Weil and Perles (1938) have recorded cases of splenic tuberculosis with intense erythroblastic reaction in the spleen and they have included these in their group of crypto erythroblastoses It is possible that they were really dealing with the fairly common condition of tuberculosis complicating chronic myeloid leukaemia (see erythroleukaemias p 172)

Therapeutic In all cases of confirmed chronic spleen tuber

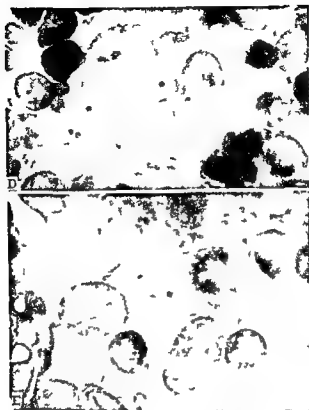


FIG 41 *d e* Syphilitic epithelioid cells

Puncture of an inguinal gland in a case of primary typhus during improvement. I owe the preparation to the kind co-operation of Dr. Ludin. (d) Young types with delicate transparent chromatin network. (e) Multi-nucleated giant cells. (Those shown are a rather older type with close chromatin.) The nuclei are somewhat larger and the cytoplasm wider than that in the epithelioid cells of tuberculosis.

bluish grey without any granules. In places it was possible to see these cells in process of forming multi-nucleated giant cell (Fig 41 e). Forteza saw only a hyperplastic reaction in an inguinal gland of a primary sore and chronic inflammation with many plasmacytes in a necrotic gland (Stuyt, Strunge, Pavlovsky). Although no spleen punctures have been recorded it seems certain that a picture similar to that in lymphatic glands, i.e. with epithelioid cells, would be found.

(d) Morbus Bang

We have records of 7 spleen punctures and 1 smear. In 5 cases (4 punctures and 1 smear) we found large reticulo-endothelial cells (Fig 41 c) which were almost certainly identical with the fusiform

a cubital lymphatic gland and the diagnosis of sarcoidosis was also confirmed by histology (Fig 47 *a b*) Stahel (1939) and Leitner (1940) had also recorded diagnosis of this disease by gland puncture. It seems reasonable to suppose that the spleen would show similar appearances.

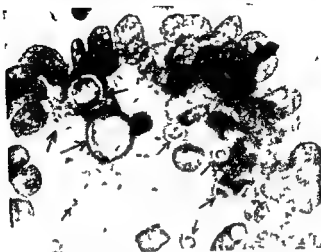
The epithelioid cells of sarcoidosis are indistinguishable from the younger ones found in tuberculosis but only well preserved ones are found there is never any sign of cellular senility such as pyknosis. This is presumably, attributable to the absence of caseation in sarcoidosis. There are, however differences of distribution in tuberculosis the epithelioid cells found in gland puncture tend to be arranged in small groups whereas in sarcoidosis their arrangement is in distinct groups or draught of fishes agglomerations. If therefore in the absence of positive tuberculin tests this distribution were seen in spleen puncture one would be prepared to make a definite diagnosis of sarcoidosis. Some of the puncture material should always be used for sections in order to demonstrate the characteristic nodules. Van Beck found such granulomata in liver puncture while Stahel and Gormsen were able to see them in sections of sternal puncture fluid.

(c) Syphilis

We have no personal observations but splenomegaly sometimes associated with syphilitic cirrhosis may occur in both the congenital and the acquired disease (Naegeli Curschmann, etc.) Inhibition of marrow function may ensue but can often be cured by anti-luetic treatment although splenectomy may be required (case of Wintrobe).

The appearances of the specific epithelioid and giant cells have not yet been recorded in the literature but Ludin did describe the characters of gland puncture in a paper read at the Swiss Hematological Society in 1948 (inguinal gland of a man aged 26 with a healing primary sore roseolar rash and positive W.R.) By his kindness we have been able to examine and photograph the films (Fig 41 *d e*) Ludin found epithelioid and giant cells but in addition there were signs of chronic inflammation with many lymphocytes showing every stage of maturation from lymphoblasts to mature cells together with many reticular elements of the type of sinus endothelium some of which showed evidence of phagocytic activity. In addition there were many plasma cells of all ages from plasmoblasts to mature forms.

Syphilitic epithelioid cells (Fig 41 *d*) closely resemble the younger ones found in tuberculosis their nuclei are rather thicker and larger with very fine chromatin in the younger cells. Unlike Hodgkin cells there is only one small bluish red nucleolus. The cytoplasm is broader than in the epithelioid cells of tuberculosis and is pale

FIG. 41 f *Blastomycosis*

Paenacodon brasiliensis Spl n I re de Almeida Disease Large giant cells which arise by fusion of epithelioid cells are here seen containing the typical fungi which are seen as the refractive spheres with a typical double outline

It would be reasonable to believe that in other fungus diseases of the spleen e.g. coccidioidomycosis the fungi could be demonstrated in the spleen puncture. To our knowledge no such reference has yet been made.

C HODGKIN'S DISEASE

We are inserting this section between those on inflammatory and neoplastic conditions because Hodgkin's disease cannot definitely be assigned to either group although on the whole American writers incline to the neoplastic theory (for literature see Ratkoczy).

The spleen is often clinically affected (Naegeli) while pathologically it is frequently enlarged and widely replaced by the characteristic granulation tissue (Lubarsch). Indeed cases in which the disease is confined to the hepatolienal system for a long time are not uncommon; and of course present much diagnostic difficulty (Pedro Pons Farreras Valenti).

Introzzi was the first to use spleen puncture as a diagnostic method while Storti (1935) Weil (1936) Heilmeyer (1942) and Farreras Valenti (1947) have also recorded cases in which the finding of the characteristic Dorothy Reed cells led to a diagnosis.

The first descriptions of the character of these cells as seen in films are due to Guthrie (1921) and Introzzi (1932) and their discovery in films from gland puncture has often been recorded e.g. Pavlovsky (1934) Weil (1936) Fleischhacker and Klima (1937) Trschendorf (1939) Storti (1939) Stahel (1939) Forteza (1947) Ludin (1948) etc.

epithelioid cells which were first described in sections by Löffler and von Albertini. We have never seen similar cells in any other condition. Further the films showed the usual signs of a chronic inflammatory reaction with normal and slightly increased numbers of lymphocytes (75-80 per cent).

Structure These cells are striking on account of their size, greyish blue cytoplasm, indistinct outline and when seen in groups absence of intercellular connections. The nucleus (17-28 μ) is longish oval or monocytoid with some lobulation. The chromatin is reddish violet and has a reticular arrangement with varying sizes of mesh (see Fig 41 c). Occasionally a small nucleolus can be seen.

We have already discussed the spleen films of a case complicated by hemolytic anemia (p. 72) in which in addition to cells of this type there were many erythroblasts and a few myelocytes.

As we pointed out in our earlier work (Löffler and Moeschlin) it is not uncommon to find a Bang infection manifesting itself by splenomegaly without any signs of general disease. It is therefore recommended that agglutination tests should be done in every case of splenomegaly of uncertain origin whether accompanied by general symptoms and lymphocytosis or not.

Our findings have been confirmed by Sundberg and Gormsen who found the characteristic cells in the sternal marrow.

Diagnosis Spleen puncture is not of value as a routine procedure but it can be so in chronic cases (see above). For example Dr Buchler (Tiefenausspital, Bern) kindly sent us films from a boy of 17 who had suffered before from a definite abortus infection and who now had anemia (hemoglobin 60 per cent) and only a weakly positive agglutination; therefore diagnosis was uncertain and other diseases had to be emphasised. The typical Bang cells were found in the films and treatment with collargol (Löffler) resulted in disappearance of all the signs.

(e) Mycoses of the Spleen (*Blastomycosis brasiliensis*)

Mycoses of the spleen are extremely rare diseases in Europe. The South American blastomycosis (*paracoccidioides brasiliensis*) or Splendore de Almeida Disease is actually the only one of practical importance. It is a generalised or localised fungus (Manson) and can also attack the spleen. We are most grateful to Dr Silva of San Paulo for such a spleen puncture which is reproduced here in Fig 41 f. The clear strong light refractory spheres with double contours of 1-30 μ in diameter are typical. All transitional stages are to be found, i.e. from minute configurations containing clear eccentrically located darkly stained nuclear shaped structures to larger vitreous spheres (M. P. da Silva 1943). Moreover the abundance of epithelioid cells, some of which fuse to form multinuclear giant cells is further characteristic of the puncture.

cate network of the younger nuclei a rather large dark blue nucleolus is present (Figs 37 and 42 *b*) Guzman using special stains in sections has called attention to differences between the nucleoli of these cells and those of normal reticulo endothelium

As the nuclei became older the chromatin becomes closer and thicker (Fig 39) while the nucleoli are usually larger deeper blue and more numerous standing out distinctly in the reddish violet of the nuclear chromatin Increasing segmentation of the nucleus is a further indication of ageing Mitosis without division of the plasma may result in the formation of multi nucleated cells (Figs 38 and 42 *a*)

The cytoplasm is relatively broad being azure blue in the younger types and as maturation increases passing from pale blue to pale violet to grey into the multi nucleated forms in which vacuolation and azurophilic granulation commonly occur

By the examination of many films we found typical mitoses of Dorothy Reed cells (see Fig 42 *f g*) Comparing their chromosomes with those of reticulum cells (Fig 23 *b e f*) the broad cytoplasm of the Dorothy Reed cells and the acute angle of the shorter and clumsier chromosomes is striking Only the basophilic forms seem to enter into division and in these forms in co operation with Thorell we could by using ultra violet absorption microscopy demonstrate large amounts of desoxy ribose nucleic acid This seems to point at the great tendency to proliferation of these young basophilic forms

We doubt the accuracy of Heilmeyer's contention that transitions from reticulo endothelial elements to typical Dorothy Reed cells can be found Equally we are far from convinced that these cells divide by amitosis as Strunge asserts in our opinion it is more probable that the nuclei divide mitotically without concomitant division of the cytoplasm hence the formation of multi nucleated giant cells (see Amitosis p 43)

Our view is that the available evidence strongly suggests the neoplastic nature of these elements and we would specially call attention to the large size of the nucleus relative to that of the cytoplasm the characters of the chromatin the presence of numerous often abnormal nucleoli and the general polymorphism of the cells The local signs of inflammation and the general signs (fever increased Σ S R) are to be interpreted as the result of a reaction to abnormal protein substances set free by degeneration of the characteristic cells Then again the fact that radio therapy produces an almost specific destructive effect on these cells (Case 4) is further evidence in favour of their neoplastic nature Of course the neoplastic hypothesis does not necessarily exclude a virus as the cause (Grand Castellani) and in a forthcoming publication Schwarz and I will discuss the subject in detail (27 A and 27 B)

We can report on several cases of gland puncture and 8 of spleen puncture

STRUCTURE OF DOROTHY REED (STERNBERG) CELLS IN FILMS

The most striking features are the variations of size of both cell and nucleus and the dark blue or violet nucleolus. Probably no other

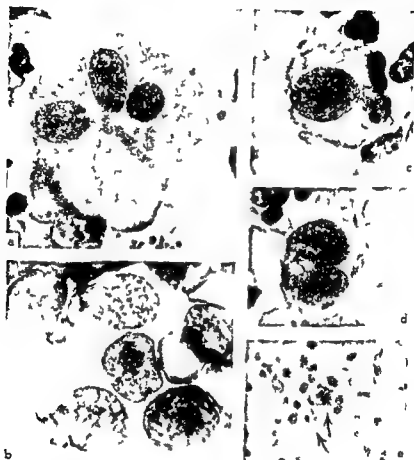


FIG. 4. Dorothy Reed (Sternberg) cells in Hodgkin's disease

- (a) Multi nucleated forms
- (b) Young types
- (c) Typical mature cells with finely granular chromatin and dark blue nucleoli
- (d) Giant-cell
- (e) Histological section from embedded spleen puncture material - Two typical Sternberg cells with dark nucleoli are seen

elements except sarcoma and carcinoma cells manifest such differences of size ($18-70\mu$). In the younger forms the nucleus ($14-24\mu$) is round or ovoid while in older cells it is up to 51μ indented, loculated or even divided into separate parts which may or may not be connected with one another. In the close but deli

In spleen puncture we occasionally saw young macrophages with a bluish nucleolus but these cells showed much smaller nuclei than those of the Sternberg cells. The only other conditions in which we have seen the abnormal cell types have been in 2 cases of retothel sarcoma but unlike the condition in Hodgkin's disease there is no associated inflammatory reaction in spleen films which showed mainly lymphocytes with a few neutrophils monocytes and plasma cells. Indeed in our opinion the absence of a concomitant inflammatory reaction should arouse a suspicion of retothel sarcoma or on other neoplasm.

The remarkable size and the basophilia of the nucleoli of Hodgkin cells indicate an intense formation of proteins and may perhaps explain the rapid proliferation of the Hodgkin's tissue. The investigations of Caspersen Thorell and Hyden using ultra violet absorption microscopy have shown that normally the precursors of cell proteins are produced by the nucleoli. These pass towards the nuclear membrane where it is supposed formation of the cytoplasm occurs. Guzman who used a special staining method reached similar conclusions.

Spleen Puncture A case in which the diagnosis was made by spleen puncture will be recorded in some detail because the absence of glandular enlargement made clinical diagnosis impossible.

Case 1 S.S. a housewife aged 65 had complained of fatigue anorexia and oppression in the upper abdomen since the beginning of 1935 (aged 59). She was admitted to Professor Clairmont's surgical clinic where an enormous smooth hard swelling of the spleen extending to the navel was discovered as well as a few soft glands the size of peas in the supraclavicular regions. Leucocytes 4200 with neutrophils 51 per cent lymphocytes 42.5 per cent and no eosinophiles platelets 99000 red corpuscles 3.8 millions hæmoglobin 78 per cent reticulocytes 1.4 per cent. The sternal marrow appeared normal.

One of the glands was excised and showed some increase of eosinophiles and reticulum cells but no Sternberg cells were found. A tentative diagnosis of aleukæmic lymphadenosis was made and radio therapy was started. The spleen diminished in size and the general condition improved greatly the patient remaining in good health until 1941.

In the spring of that year she was again tired with a temperature of about 100. She again entered hospital in October.

Poor general condition Small soft glands the size of peas. Liver not enlarged. Spleen 13 cm palpable firm and smooth. Blood hæmoglobin 69 per cent reds 3.7 millions reticulocytes 3.7 per cent occasional normoblasts. Leucocytes 2400 with myelocytes 2.5 per cent staffs 26 per cent polymorphs 22.5 per cent monocytes 26 per cent lymphocytes 22.5 per cent no eosinophiles. Platelets 105000. The neutrophile granules were rather coarse. Sternal and iliac crest puncture showed slight immaturity of myelopoiesis but no Sternberg cells. B.S.R. 2 mm Takata negative Hypoproteinaemia 4.8 mg per cent Prothrombin 50 per cent and no rise after Synkavit. Serum iron 120 gamma. *Spleen puncture* many typical Hodgkin cells (see Fig 42 b). For splenogram see Table 20 Case 1. Death ensued and



FIG 42 *f g* Typical mitoses (*f*) prophase (*g*) monaster) of Dorothy Reed Sternberg cells. In these mitoses the broad cytoplasm and the acute angle of the shorter and clumsier chromosomes is striking compared with those of reticulum cells (Fig 23 *b = f*)

The typical elements differ from the epithelioid cells of tuberculosis in their possession of bluish violet nucleoli and the blue cytoplasm of the less mature forms. The older multi nucleated forms show some resemblance to megakaryocytes (Fig 42 *d*) but can be distinguished by the structure of their nuclei and especially by the characteristic tint of the nucleoli.

diagnosis was confirmed by autopsy in the other by the histology of an excised gland

In Case 4 in which the diagnosis had been made histologically spleen puncture performed 6 months after the end of a course of radio therapy failed to reveal any Sternberg cells. There were however many eosinophiles (45 per cent as compared with the normal 2-15 per cent) together with 5 per cent of normal monocytes (see Table 20)

Our other cases also showed some degree of chronic inflammatory change viz distinct monocytosis increase of pulp cells and shift to the left of the neutrophiles. Large lymphatic plasma cells were slightly increased but we never found excess of plasmacytoid reticulum cells. Eosinophiles vary from case to case and in different stages of the disease as Ludin also found in gland punctures indeed eosinophiles may be absent

Diagnostic Value of Spleen Puncture. Our findings support the views of other writers that spleen puncture can be of great diagnostic aid in Hodgkin's disease. In fact if only the spleen is affected and no other means of early diagnosis is available and it may clinch matters in cases in which the histology of excised glands (and gland puncture) gave no clear results

The typical cells are not often found in the sternal marrow (Klima Kienle Paula e Silva). Indeed although sternal puncture is performed as a routine in cases of Hodgkin's disease at the Zurich clinic we have only once been able to demonstrate Dorothy Reed cells. This is doubtless due to the fact that involvement of the marrow usually occurs only in the late stages and is even then patchy. The spleen on the other hand is often affected very early and puncture is therefore likely to give a positive result

We agree with Tischendorf that the details of Hodgkin's cells are more normally visible in films than in sections of excised glands. This is specially important in those early cases in which the histology is simply that of an apparently unspecific over growth of reticulum. For this reason if excision of a gland is performed for diagnosis films from the cut surface cells as well as sections should be examined. In the former the younger Sternberg cells are more easily distinguished from other reticular elements. Of course we do not suggest that examination of films should entirely replace sections the two methods supplement one another

D NEOPLASTIC DISEASES OF THE SPLEEN

XI HÆMOBLASTOSES (LEUKÆMIAS)

We cannot discuss the modern use of the pathogenesis of the leukæmias here which has been considered in an earlier work

autopsy (Pathological Institute . Professor v. Meyenburg) showed Hodgkin's disease in the spleen and liver as well as in the medullary spaces of the pelvic bones and ribs

There was thus a case of intermittent pyrexia with anæmia leucopenia hypoproteinæmia and splenomegaly in which the initial good effect of radio therapy led to suspicion of a neoplastic disease such as reticulosis or aleukæmic leucosis. The low B S R was taken as evidence against a diagnosis of Hodgkin's disease while the hypoproteinæmia was attributed to infiltration of the liver and perhaps of extensive areas of the bone marrow. As the spleen puncture showed unexpectedly Dorothy Reed cells the diagnosis was at once cleared

TABLE 20
Hodgkin's Disease

Case number	1	2	3	4
Age sex	65 ♀	26 ♀	49 ♀	39 ♂
Size of spleen (in cm)	13	17	12	—
<i>Hæmogram</i>				
Hæmoglobin ()	69	64	37	—
Leucocytes	2 400	2 200	10 900	—
Neutrophiles staff	26	60½	16½	—
Neutrophiles segmented	22½	14½	16½	—
Eosinophiles	—	½	—	—
Basophiles	½	—	—	—
Monocytes	26	18½	8½	—
Lymphocytes	2½	4½	13½	—
Plasma cells	—	½	—	—
<i>Splenogram</i>				
Macrophages	0.1	0.2	0.9	—
Plasmacyt retic cells	0.4	0.2	0.1	0.3
Pulp cells	2.1	4.1	0.6	0.7
Erythroblasts	0.2	—	—	—
Myelocytes	1.0	0.6	—	0.3
Neutrophiles staff	6.9	31.6	3.8	7.3
Neutrophiles segmented	6.2	13.2	13.7	10.7
Eosinophiles	—	1.9	1.1	4.6
Basophiles	0.1	0.2	0.2	—
Monocytes	6.1	9.5	3.3	5.0
Lymphoblasts	—	0.2	—	—
Lymphocytes small young	6.4	1.7	0.6	4.7
Lymphocytes small old	63.8	24.7	70.0	60.0
Lymphocytes large young	2.5	1.0	—	1.3
Lymphocytes large old	2.4	7.4	0.9	3.7
Lymphocytes total	75.1	35.0	71.5	69.7
Plasma cells	0.5	0.5	0.3	1.4
Total Hodgkin cells	1.3	3.0	4.5	—

In the case of a woman of 26 (Fig 42 c) there was leucopenia and anæmia while in a woman of 49 (Fig 42 a d e) there was neutrophile leucocytosis and lymphocytopenia. In both cases spleen puncture revealed many typical Sternberg cells: in one the

phatic leukæmia unless a good deal of blood has become mixed with the spleen fluid

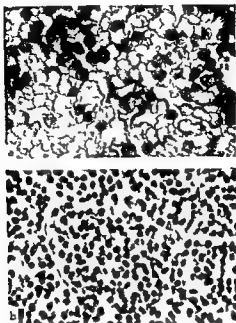


FIG. 43 Lymphatic leukæmia (aleukæmic lymphadenosis of the spleen)
 (a) Film from spleen puncture purely lymphocytic
 (b) Section from spleen puncture

A striking view of spleen films in lymphatic leukæmia is the almost complete absence of pulp cells and a similar state of affairs is found in the lymphosarcoma follicular lymphoblastoma and in some reticulosés. Histologically practically no trace of the sinuses can be seen in sections because the proliferating cells practically obliterate them. The structural differences between the various cell types are discussed later.

Our observations suggest that it is unnecessary for diagnostic purposes to carry out detailed differential count of the cells in spleen films. Careful examination will give an idea of the approximate distribution and will show the finer details. If there is suspicion of lymphatic leukæmia a differential count of 500 cells is advisable and should never be omitted in doubtful cases in which lymphocytes are plentiful in the film.

There is one important source of error in such counts. It is known that the lymphocytes in blood films in lymphatic leukæmia are structurally delicate which leads to the presence of ruptured cells (Gumprecht shadows) scattered throughout the preparation. This is of course an indication of the essentially abnormal character

(Moeschlin and Rohr (3)) in which the leukemias were discussed as neoplastic diseases

Our observations on spleen puncture in these conditions have been divided into the following sub groups —

- (1) Lymphatic leucoses
- (2) Myelocytic leucoses
- (3) Paramyeloblast leucoses
- (4) Polycythemia vera
- (5) Erythroleucoses and erythroblastoses
- (6) Reticuloses

Diagnostic Significance Spleen puncture is mainly of diagnostic value in aleukemic cases (Weil Storti Montes) in which this procedure quite often permits recognition of the disease in most obscure cases *(especially of chronic aleukemic lymphatic leucoses)*. It can also be of diagnostic importance in cases of myeloid reactions in which sternal puncture may not enable a diagnosis to be made either because there is no bone marrow e.g. in osteosclerosis or because in initial cases no characteristic changes have yet developed in the bone marrow. For instance we have already mentioned (p. 61) the case in which well marked clinical inflammatory splenomegaly was accompanied by persistent leucocytosis of 20 000 with a few myeloid cells and only a spleen puncture did permit a definite diagnosis.

An interesting sidelight into the details and progress of cases of leukemia can be obtained by spleen puncture which also permits control of treatment and closer observation of the course of mitosis.

1a CHRONIC LYMPHATIC LEUCOSES

(Leukemia)

Spleen puncture shows great predominance of lymphocytes usually with many immature forms (Weil 1936 Storti Lopez and Montes 1939). We have investigated 18 cases of which 9 (4 aleukemic and 5 leukemic) are described here.

In all these cases the possibility of paramyeloblastic leukemia can be excluded because of the clinical course and the characters of the sternal marrow.

Lymphocytes in Spleen Puncture

The lymphocytes vary between 92 and 99 per cent which is higher than in any other condition except one case of Felty's disease in which we found 90 per cent of lymphocytes. *The presence of more than 95 per cent of lymphocytes therefore permits an almost definite diagnosis of lymphatic leukemia*. If however they are in the region of 90 per cent the characters of the cells and the whole clinical picture must be taken into account. Figures below 90 per cent in untreated cases practically exclude the diagnosis of lymphatic

Lymphatic Leukæmias

5	6	7	7	8	9
600	785	650	650		640
(b) leukæmic cases					
54	84	83	83	—	88
47 500	1.5 000	10 300	13 800	—	20 100
97	911	881	90	—	781
40 075	114 375	179 4	17 470	—	15 778
40	15	27	27	?	11
++	+++	—	—	?	—
—	0.1	—	+	—	—
—	—	—	—	0.5	—
+	+	—	—	—	—
—	—	—	—	0.7	—
0.1	0.1	—	+	—	0.1
0.5	0.5	0.3	0.3	1.2	0.3
0.5	1.9	0.5	0.5	0.7	1.2
—	0.2	—	0.1	0.1	—
—	0.1	—	—	—	0.1
0.3	0.1	0.2	0.1	0.2	0.2
—	—	—	—	—	—
—	—	—	—	—	—
0.4	1.1	—	—	—	0.8
3.0	6.6	3.0	2.3	11.8	9.4
93.7	83.1	94.9	93.8	64.3	65.7
1.4	4.0	0.4	0.2	13.9	1.4
0.9	2.1	0.7	0.7	6.6	1.7
98.9	96.9	99.0	99.0	96.6	98.0
4.4	10.6	3.4	2.5	15.7	30.8
—	1	—	—	—	1
small	extremely	small	uniform	almost	mainly
immature	polymorphic	old	fragile	normal	young
fragile	fragile	lymphocytes		lymphocytes	and fragile
43	48	59	65	90	76

seen in normal lymphocytes their occurrence should therefore always arouse a strong suspicion of lymphatic leukæmia

It is not only the great predominance of lymphocytes that characterised lymphatic leukæmia (Fig 43 a) the frequency of young cells is striking in most cases (Weil Storti Montes) Normally such elements never exceed 10 per cent but a glance at Table 21 reveals their increase in lymphatic leucoses Only in inflammatory diseases and in lymphatic reactions is there a similar increase but of course associated with other indication of inflammation Admittedly we did not observe any immaturity of the lymphocytes in 4 cases of lymphatic leukæmia in which the cells were strikingly small and old

Weil and Montes called attention to the presence of lymphoblasts but as our cases show this is inconstant perhaps however they defined lymphoblasts differently On the whole it can be

TABLE 21

Case number	1	2	3	4
Age sex	49 ♀	47 ♀	50 ♂	56 o
(a) aleukæmic cases				
<i>Hæmogram</i>				
Hæmoglobin (°)	65	90	9	89
Leucocytes	1 200	2 800	6 900	7 500
Lymphocytes ()	66½	22	29	51½
Lymphocytes absolute	798	616	2 001	3 870
Spleen (in cm)	20	15	16	13
Palpable glands	—	—	+	+
<i>Spl noogram</i>				
Macrophages	0.1	—	0.1	+
Plasmacyt retic cells	0.5	+	1.1	+
Pulp cells	0.1	0.1	—	+
Erythroblasts	0.3	—	0.9	—
Myelocytes	—	—	0.1	—
Neutrophiles staff	1.3	1.6	0.5	0.9
Neutrophiles segmented	0.8	4.9	1.8	3.3
Eosinophiles	0.1	0.5	1.0	0.5
Basophiles	—	0.3	0.1	—
Monocytes	1.2	0.5	0.6	0.3
Plasmoblasts	—	—	0.1	—
Plasma cells mature and half mature	0.2	0.1	1.2	0.2
Lymphoblasts	—	0.1	2.4	0.4
Lymphocytes small young	1.1	0.6	19.8	17.0
Lymphocytes small old	86.7	84.4	64.7	67.1
Lymphocytes large young	0.8	0.5	2.9	7.9
Lymphocytes large old	6.8	6.3	2.6	2.4
Lymphocytes total	95.4	91.9	97.4	94.8
Lymphocytes total young forms	1.9	1.1	22.7	24.9
Mitosis per 1 000 cells	—	+	3	1
Character of cells	half mature small fragile	uniform very fragile	very poly morphic very fragile	very poly morphic very fragile
Puncture number	9	17	57	47 III

of these elements. In preparation of spleen films similar shadows are numerous and their presence is suggestive of the diagnosis of lymphatic leukæmia. It is therefore essential to choose the best parts of the films for a differential count.

Spleen puncture usually supplies plenty of material mixed with very little blood in cases of lymphatic leukæmia and the greyish yellow colour of the fluid immediately arouses suspicion of lymphatic or chronic myeloid leukæmia or of lymphosarcoma. Sections of embedded puncture material show the gross increase of lymphocytes equally as well (Fig 43 b).

Structure. These lymphocytes show a striking arrangement of the nuclear chromatin into focal masses (Fig 44 a) in almost all cases of lymphatic leucosis. Strunge mentions a similar state in the cells of gland puncture (*etat grumel*). Another common feature is slight lobulation or transverse indentation of the nuclei such ■ are rarely

morphism and those with many lymphoblasts than in those with predominance of older types of small cells

The other cellular elements in the spleen showed some changes. Thus in about half the cases there were isolated erythroblasts and myelocytes but we never found any increase of plasmacytoid reticulum cells. In Case 3 there was slight excess of lymphatic plasma cells (1·2 per cent) and also fairly numerous tissue mast cells (0·2 per cent). Unlike the condition found in lymphatic reactions pulp cells are very rarely found in the films.

Diagnostic Significance (1) Aleukæmic Cases

Spleen puncture is obviously most likely to be of value for diagnosis in those cases in which there are no glandular swellings and sternal puncture shows no excess of lymphocytes. Such cases in which only the spleen and liver are affected at least in the early stages of the disease are by no means uncommon; indeed Cohnheim placed them in a group separate from the other lymphadenoses. Cases 1 and 2 were of this type and one is recorded here in some detail.

Case 1 Housewife aged 49. Complaint of fatigue, loss of weight and development of pigmented spots in the extremities. Splenic enlargement was detected in 1938. In 1939 eruptions occurred again on the hands and in 1940 she went to the Dermatological Clinic where splenomegaly, leucopenia and a positive Wassermann reaction were found. She was then referred to the medical clinic.

March 7th 1940 poor general condition (45·1 kilos). No enlarged lymphatic glands. Liver easily palpable, firm and smooth. Spleen (20 cm) firm, smooth and extending to the navel. *Blood picture*: hæmoglobin 74 per cent, reds 4·4 millions, reticulocytes 1·5 per cent, leucocytes 1·200, sticks 2·5 per cent, polymorphs 16 per cent, monocytes 14·5 per cent, lymphocytes 66·5 per cent (800 per cmm). *Sternal puncture*: slight inhibition of myelopoiesis, no percentage increase of lymphocytes, nothing suggestive of myeloid leukaemia. Histamine fast achylia gastrica. BSR 65, WR ++, Takata ++, Weltmann 0·5. Serum protein 8·5. Prothrombin time 24 seconds (normal 13). Low serum iron (54 gamma). Bilirubin not increased. Congo red test for amyloid negative. Cholesterol normal (169). Galactose negative. Diastase normal. Osmotic resistance of reds normal.

In brief then this is a case of well marked splenomegaly with leucopenia (1·200) and reduction of lymphocytes to 800 without any enlargement of lymphatic glands. The positive Wassermann reaction, increased sedimentation rate, augmented blood protein level and the achylia aroused a suspicion of syphilitic cirrhosis which is a condition that may be accompanied by great enlargement of the spleen (Naegeli (2)) but the possibility of an aleukæmic leukaemia or some other form of splenomegaly was borne in mind.

Sternal puncture showed slight shift to the left to myelopoiesis.

said that lymphoblasts are most numerous in cases with cellular polymorphism which we found in only 9 of our 18 cases. Variations in size, nuclear indentation, number and size of nucleoli and breadth of cytoplasm can be seen in the peripheral blood in some cases of lymphatic leukemia but it is even more striking in spleen films (Fig 44 a) in which lymphoblasts also show these peculiarities. On the whole cases with cellular polymorphism run a more malignant course than those with predominance of small old lymphocytes.

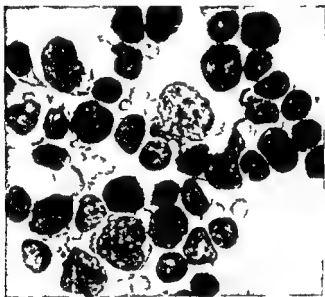


FIG 44 a Lymphatic leukemia

(a) Variations in size of cells, some of young type. See striking arrangement of the nuclear chromatin into focal masses.

If there is great polymorphism, intense basophilia of the cytoplasm, delicate nuclear structure with numerous large nucleoli and above all, increased blood sedimentation, the possibility of lymphosarcoma must be remembered (see p 128).

The cells of reticulososes usually possess closer and more compact chromatin (Fig 51) while those of follicular lymphoblastoma have delicate indentations of the nucleus (Fig 53 d e). These features may, however, be absent, and then diagnosis will depend upon the general clinical condition.

Our view is that histological diagnosis is even more difficult than cytological because of shrinkage due to fixation.

The problems associated with mitosis have already been discussed (p 40). All that need be said here is that cell divisions are more numerous in the cases of lymphatic leukemia with cellular poly-

young basophilic forms (glandular fever) and by the always transient reaction (acute lymphocytosis). The lymphocytes in spleen puncture in these conditions even when the glandular fever cells are included never rise above 85 per cent.

The Relationships of Lymphatic Leucosis to Reticulosis

The reticuloses will be discussed in a separate section but it will be convenient to discuss our opinion that many so-called lymphatic leucoses especially the aleukemic forms should be included among the reticuloses. It may be assumed that the neoplastic degeneration in these cases affects some element even less differentiated than the lymphoblast viz. a reticulo-mesenchymal cell. But admittedly these elements cannot always be distinguished by their structure from the atypical lymphocytes of lymphatic leucoses. Of course if Gitterfasern can be seen there is no doubt as to the diagnosis but in many reticuloses the power of forming fibrillar reticulum has been lost. Even so we have reached the conclusion that some cases of aleukemic or sub-leukemic lymphatic leucoses (especially those with increased sedimentation rate) are really reticuloses especially because in some cases the increased sedimentation rate is associated with the presence of abnormal plasma globulins or a raised serum protein level similar to the state of affairs in myelomatosis. The Takata or cephalin reaction may also be positive in these circumstances while Wuhrmann and Wunderly have demonstrated abnormal electrophoretic curves.

In Case 1 the serum protein was 8.5 mg per cent the Takata reaction was positive the blood sedimentation was 61 mm in one hour and there was a probably non specific positive Wassermann reaction. In a man of 67 (Case 3) the B.S.R. was 67 mm serum protein 8.5 mg per cent and the Takata reaction was positive. In a man of 33 the B.S.R. reached the remarkable figure of 89 mm with a positive Takata reaction and a serum protein level of 9.2 mg per cent (see Fig. 50 d).

1b ACUTE LYMPHATIC LEUCOSES

Careful consideration of cases in our Clinic has convinced us (Moeschlin and Rohr (3)) that most of the cases recorded under this name in the literature have really been micromyeloblastic. For example we have often seen chronic myelocytic leucoses which terminated as micromyeloblastic leukaemias in which the predominant cells were identical with those regarded as typical of acute lymphatic leucosis by Wintrobe (see his Plate XI B p. 698).

We would confine the term acute lymphatic leucoses to those cases in which the predominant cells in the blood closely resemble lymphoblasts while in the early stages the disease is confined to the

but no increase of lymphocytes. Spleen puncture (Table 21 Case 1) revealed the diagnosis immediately because there were 95.4 per cent of lymphocytes typical of an aleukæmic lymphatic leukaemia and quite unlike any inflammatory disease of the spleen. Death occurred 6 months later as a result of a lung abscess and the post mortem showed typical lymphadenosis of the liver and spleen but with no glandular enlargement except in the mesentery. It seems possible that the Wassermann was probably a non specific result of the changes in the blood proteins.

In Case 2 a woman aged 47 there was great enlargement of the liver (23 cm.) and distinct splenomegaly (15 cm.) without any associated glandular enlargement. There was leucopenia (2,800) with only 22 per cent of lymphocytes. The serum protein was low (5.9). Takata reaction was negative and the blood sedimentation was low (3 mm.) in this case also spleen puncture showed striking fragility of the lymphocytes (Table 21 Case 2). 6 months later there was slight enlargement of some supraclavicular glands.

These 2 cases emphasise the difficulty of diagnosis in cases of aleukæmic lymphosclerosis in which there is splenomegaly but no glandular enlargement. In Case 1 the association with an infective process increased the difficulty of differential diagnosis and a number of diseases had to be considered. We only mention Hodgkin's disease and chronic tuberculosis of the spleen both of which may be impossible to exclude without spleen puncture. Diagnosis is of some practical significance because the prognosis in such cases of chronic aleukæmic lymphadenosis is relatively good.

In Cases 3 and 4 there was enlargement of lymph glands as well as of the spleen and liver so that the diagnosis could be made by gland puncture. In both these cases spleen puncture showed the presence of many young lymphocytes with considerable polymorphism.

(2) Sub leukaemic and Leukaemic Types

Spleen puncture is really of little importance in these types of lymphadenosis because the typical enlargement of lymphatic glands together with an increase in the absolute numbers of lymphocytes makes a diagnosis obvious except in the rare cases in which only the spleen is involved. Then spleen puncture is recommended even if the number of lymphocytes in the peripheral blood is high so as to avoid confusion with lymphatic reaction of non leukaemic nature. We have already mentioned such a reaction accompanied by splenomegaly and associated with a pituitary tumour (p. 91). In this case a diagnosis was obvious from the character of the spleen films because there was no great degree of lymphocytosis (under 90 per cent). Lymphatic reactions due to infections can be distinguished more easily from lymphatic leukaemias by the appearance of large

cells with broad palely basophilic cytoplasm. The nuclei were rounded or elongated with an arrangement of the chromatin closely resembling that of lymphoblasts containing several nucleoli some of which were large (Fig 44 b) mitoses were numerous. The excessive numbers of young and abnormal cells was unlike the condition found in an ordinary lymphadenosis and a diagnosis of (probable) lymphosarcoma was made.

Histologically the spleen structure was almost obliterated and no follicles could be recognised. The great cellularity of the pulp led to a suspicion of myeloblastic leukaemia (Pathological Institute University of Zurich Professor von Meyenburg).

The immediate post operative course was satisfactory.

Sternal puncture showed normal myelocytic marrow with no pathological cells. The blood picture was normal for 2 months.

A month after the operation a small firm nodule developed in the scar followed by enlargement of the right supraclavicular glands and then by axillary ones. Gland puncture revealed cells of the same type as in the earlier spleen films. Two months after the operation the leucocytes suddenly rose to 65 800 with 15 200 erythroblasts 2.6 per cent of half mature and mature myelocytes and 60 per cent of lymphatic cells partly of paralymploblastic type (Fig 44 c). There were a few paralymploblasts with coarse azurophilic granules similar to those of mature lymphocytes.

The liver was enlarged. Treatment with arsenic and later with urethane caused gradual improvement and decrease of leucocytes but a few paralymploblasts could still be found in the blood. The B.S.R. was persistently low (3-6 mm). There were only a few abnormal cells in the sternal marrow probably due to admixture of blood otherwise erythropoiesis was normal and leucopoiesis was of the ordinary myelocytic type.

The patient left hospital at his own wish. Large masses of glands developed and death occurred soon afterwards.

Summary. This was a typical case of lymphosarcoma of the spleen in a man of 50 in whom glandular enlargement and blood changes became manifest 2 months after splenectomy (with about 30 000 per cmm (60 per cent) of paralymploblasts and paralympocytes). We were thus able to follow all the developmental stages of a genuine acute lymphatic leukaemia originating in the spleen. Thus at first there was an aleukemic phase with localised lymphosarcoma and then generalisation with involvement of lymphatic glands and probably of the liver together with the appearance of tumour cells in the circulation *i.e.* the leukaemic stage.

The paralymploblasts showed some signs of a tendency towards maturation into paralympocytes (Fig 44 c) sometimes with the presence of coarse azurophilic granules in the cytoplasm. There is thus no doubt of the lymphatic nature of the affected cells and of the close relationship of lymphosarcoma to acute lymphatic leucosis (see also Hauswirth).

It is interesting to find that the bone marrow was not affected until the terminal stage and that urethane had a transiently beneficial effect.

spleen and lymphatic glands. Of course later the marrow may be affected and diagnosis be impossible. In children acute lymphatic leucoses are certainly commoner than in adults (Willi Heilmeyer).

Our conception of acute lymphatic leucosis is thus one of lymphosarcoma with emigration of the abnormal cells into the blood, hence the leukæmic blood picture. The following case is instructive in this connection —

F. A. Father died of carcinoma of the œsophagus. One brother of an unidentified tumour on the back.

Complaint of oppression in left upper abdomen of about 3 years duration. On November 23rd 1946 sudden and repeated pain in this region was followed by admission to hospital on November 24th 1946 with a tentative diagnosis of acute pancreatitis.

There was a firm mass in the upper abdomen with considerable guarding. B. S. R. 35 mm. Leucocytes 5600 with 24 per cent of normal lymphocytes. Laparotomy was performed because of the probability of malignancy but the mass was found to be a greatly enlarged spleen which was removed (1.8 kg).

In films from the spleen there was predominance of large polymorphic



FIG. 44. *h, c. Acute lymphatic leukemia or perhaps more accurately leukæmic lymphosarcomatosis.*

(*h*) Extremely polymorphous cells with large nucleoli and occasional azure granules (from spleen puncture).

(*c*) Rather more mature types in the blood still showing large nucleoli. The cytoplasm of some of the cells shows a pink stain inclusions (★) in addition to the normal azure granules. One normal lymphocyte is present.

from 17 per cent to 61 per cent while every type of transitional element between myelocytes and segmented neutrophils can be recognised (Fig 45). In some cases but by no means in all eosinophil and basophil myelocytes are prominent.

In those cases in which the blood contains a very great excess of polynuclears (the leucémie à polynucléaires or Cryptoleucémie of Weil) there are far more immature cells in the spleen than in the blood. Incidentally we would cavil at Weil's terminology because in his cases there were up to 20 per cent of myelocytes in the blood.

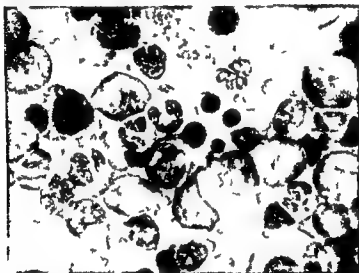


FIG. 45. Typical spleen film in chronic myeloid leukaemia with predominance of myelocytes and mature neutrophils together with many erythroblasts.

The proportion of myelocytes to more mature neutrophils appears to possess prognostic significance. Thus cases in which the number of mature granulocytes exceeded that of the myelocytes appeared to run a more chronic course; but such a state of affairs may also indicate that the disease is of more recent onset. On the other hand cases in which the number of myelocytes equalled that of more mature granulocytes or exceeded them had a distinctly worse prognosis. It is reasonable in such circumstances to assume that the disease has been of longer duration and is approaching its terminal stage. Serial spleen punctures will doubtless throw further light on this subject.

Myeloblasts are usually scanty (0.5–1.5 per cent) but higher figures indicate a bad prognosis. In all cases in which the myeloblasts were between 2.4 per cent and 17.5 per cent death occurred within a few weeks or at the longest in 9 months. Values of 10 per cent or more usually indicate the commencement of a terminal

2 MYELOCYTIC LEUCOSES (CHRONIC MYELOID LEUKAEMIA)

Terminology Before sternal puncture permitted a clear insight into the course of the myeloid leukemias two types were recognised viz, *chronic* myeloid leukemia with a blood picture in which myelocytes were numerous together with every form transitional between them and fully mature granulocytes and an *acute* disease in which paramyeloblasts and an 'hiatus leukemicus' (Naegeli (1)) were characteristic i.e. signs of cell maturation were absent. We now know that the course of such cases of paramyeloblastic leukemia may extend over several months or even years (Moeschlin and Rohr (3) Moeschlin (7) Hemmeler). The old distinction into chronic and acute forms is, therefore an erroneous one and we now speak of the former as myelocytic leucoses and of the latter as paramyeloblastic ones. It is to be understood that the name 'paramyeloblast' is applied to all those abnormal cells, granular or non granular of the myeloid series that manifest only abnormal maturation or none at all.

In myelocytic leucosis there is complete disappearance of the normal structure of the spleen as a result of dense infiltration by myelocytes and more mature granulocytes (Lubarsch). Erythroblasts and megakaryocytes can always be found but lymphocytes are scanty and true follicles cannot as a rule be recognised.

Spleen puncture confirms the histological picture inasmuch as the predominant cells are myelocytes and elements derived from them so that the films closely resemble those of bone marrow (Nagy 1924 Weil 1936 Storti and Lopez 1939).

Spleen Puncture We have records of 16 cases in which spleen puncture has been performed and in 7 this was done several times in order to follow their course and also the effects of treatment. Three cases were erythroleukemias.

The main changes in the blood and spleen are shown in Table 22.

As in lymphatic leukemias spleen puncture supplies plentiful almost bloodless grey marrow like material from which films very rich in cells and resembling marrow can be made. Quite often the small lymphoid reticulum cells described in Part I can be recognised among the granular elements whereas in most other spleen films they cannot be distinguished with certainty from the lymphocytes (see Fig. 13).

Leucopoietic Series Lymphocytes which form 60 per cent to 85 per cent of the cells in normal and chronic inflammatory spleen films are scanty and may even almost disappear (1.4 per cent in Case 1) while even during a remission due to arsenic urethane or X rays they do rarely rise above 30 per cent (Case 6).

The films are dominated by myelocytes and neutrophils the former varying from 8 per cent to 51 per cent and the latter

	(68)	(107)	(94)	(1)	(70)	(8)	(116)	(118)	(71)	(12)	(130)	(99)	(18)	(72)	(97)	(104)	(108)	(145)
<i>Splenogram</i> (No.)																		
Macrophages	—	—	—	05	—	+	01	+	+	+	—	—	—	01	—	—	01	—
Plasmacytic retic cells	—	—	—	01	03	03	03	11	—	0	02	—	—	01	03	+	12	01
Pulp cells	—	02	—	02	—	01	—	01	—	—	02	—	—	—	—	02	08	01
Erythroblasts																		
Total	66	48	72	10	74	11	134	158	21	118	453	132	152	332	592	450	408	535
Basophilic	06	08	1	01	03	03	15	29	03	14	41	08	100	729	174	95	96	53
Polychromatic	31	36	50	04	45	08	87	117	08	69	48	99	48	65	347	321	94	441
Orthochromatic	29	04	10	05	26	—	32	12	10	35	164	25	04	38	71	34	18	41
Mycloblasts	24	17	24	02	04	—	05	02	175	17	07	43	21	46	43	33	14	—
Mycelocytes total	512	501	344	371	376	84	29	109	397	374	85	453	317	221	111	80	126	52
Mycelocytes immature																		
Neutrophile	109	68	14	10	36	10	11	10	47	66	04	66	30	41	7	16	21	08
Eosinophile	01	02	01	—	05	—	02	—	—	—	—	01	06	—	01	03	—	—
Basophile	—	—	—	—	—	—	—	—	—	—	—	—	01	—	—	—	—	—
Mycelocytes mature																		
Neutrophile	218	155	99	91	67	8	38	18	149	92	18	83	58	48	29	28	8	19
Eosinophile	02	04	05	—	06	—	01	08	—	—	03	10	33	05	13	07	02	—
Basophile	—	01	—	—	—	—	—	—	—	—	01	—	04	07	—	—	—	01
Mycelocytes mature																		
Neutrophile	30	107	93	38	91	72	52	33	69	89	16	150	40	28	22	06	25	14
Eosinophile	06	06	—	—	19	01	03	01	06	02	02	23	25	12	08	09	04	—
Basophile	—	01	—	—	—	—	—	—	—	—	15	—	61	30	02	01	04	01
Metamyelocytes	146	157	132	182	152	3	122	39	126	125	76	120	69	30	07	10	44	09
Mycelocytes—mitoses (per 1 000)																		
Neutrophiles	2	1	2	—	2	—	1	1	2	2	—	1	—	—	—	—	1	—
Staff	224	236	26	369	96	160	751	11	28	232	84	108	182	137	8	60	91	42
Segmented	104	66	150	29	88	449	120	149	105	118	111	60	111	87	18	52	60	89
Eosinophiles mature	11	06	35	02	17	02	22	63	—	13	14	39	56	16	18	73	09	89
Basophiles mature	40	33	36	06	26	60	30	47	26	4	16	82	90	91	92	91	08	02
Monocytes	05	11	08	17	01	40	08	24	01	02	08	—	—	01	—	04	17	02
Lymphocytes total	14	80	104	34	115	189	197	315	47	100	218	83	71	71	95	154	246	75
Plasma cells	—	—	—	01	—	01	—	—	—	—	—	—	—	—	—	01	—	—
Megakaryocytes	+	+	+	01	+	+	+	—	—	—	+	+	—	01	+	—	+	01

TABLE 22
Chronic Myeloses

Case number	1	2	3	4	5	6	7	8	9	10	11	12	13
Age	64	34	34	60	48	34	75	66	44	33	42	61	54
Sex	♂	♀	♀	♀	♀	♂	♀	♀	♀	♀	♀	♀	♀
Duration of symptoms (in years)	3	1½	3	17	2	1	5	3½	2	4	1-2	6	2-5?
Size of spleen (in cm.)	13	20	13	15	8	15	33	25	34	27	24	20	42
<i>Hemogram</i>													
Red corpuscles (in mils.)	11/9/1942	6/6/1944	9/9/1943	4/9/1936	12/7/1940	2/11/1944	30/7/1940	23/3/1940	26/2/1944	9/5/1940	22/3/1944	3/5/1944	27/2/1946
Erythroblasts (per 100 leucocytes)	3-60	4-440	4-850	1836	3-860	5-230	—	4-200	2-840	1-490	3-140	3-670	6-200
Hemoglobin (%)	11	91	101	31	—	2	11	11	rarely	18	22	6	60
Leucocytes (in 1000s)	53	96.9	80.2	426.4	144.0	81.2	62	80	60	27	64	67	118
Myeloblasts	3	1	1	11	—	—	400.0	112.8	151.2	37.2	13.4	10.4	15.0
Myelocytes	—	—	—	—	—	—	17	—	4	3	3	2	(1)
Total	578	74	23	16	15	16	281	24	33	14	6	10	4
Immature	18	4	3	11	—	4	5	3	—	5	—	1	2
Half mature	24	7	14	2	—	2	11	9	16	4	—	2	1
Mature	48	21	11	1	—	5	7	1	3	1	—	3	—
Metamyelocytes	11	10	4	11	—	4	4	10	4	3	—	4	—
Neutrophils	—	—	—	—	—	—	—	—	—	—	—	—	—
Staff	—	—	—	—	—	—	—	—	—	—	—	—	—
Segmented	—	48	49	15	55	41	40	52	23	29	18	52	27
Basophiles (myelocytes and mature)	41	16	20	62	21	31	11	15	20	10	20	20	59
Eosinophiles (myelocytes and mature)	5	4	2	1	6	3	1	2	13	28	30	3	6
Monocytes	4	—	1	—	1	11	1	1	3	11	11	1	—
Lymphocytes	—	3	1	2	1	4	—	3	1	—	—	6	6

films is irregular perhaps because their large size interferes with their being separated from their connections. If a sufficient amount of material is obtained for the preparation of histological sections it is easy to see how much more numerous these cells are than in the case of films (Fig 46 b). It is as equally true of the sternal marrow in which megakaryocytes are approximately 10 times more numerous in sections than in films (Moeschlin (6)). Such cells may be extremely numerous in the so-called megakaryocytic leucoses (see later).

The Cause of Occurrence of Erythroblasts and Megakaryocytes
Most authors such as Naegeli, Forkner, Apitz and others assume that all the cellular systems of myeloid tissue are involved in chronic myelocytic leukemia. Formerly we have suggested the possibility that the erythroblasts and megakaryocytes which appear in the spleen and other organs in myelocytic leucoses arise as a compensatory phenomenon which to some extent makes good the damage to erythropoiesis and platelet formation due to compression by proliferating granulocytes in the marrow. This state of affairs is well seen in paramyeloblastic leucoses where oxydase negative paramyeloblasts can be seen lying close to typical erythroblasts and oxydase positive myelocytes in the liver and spleen (Moeschlin (1)). Such a proof is not possible in myelocytic leucoses because all the cells are oxydase positive.

The occurrence of chronic myelocytic leucoses in which the formation of megakaryocytes is particularly striking (the so called megakaryocytic leukæmias described by Downey, Nordland, McDonald and others) and also of erythroblastic leukæmias (in which erythroblasts are greatly increased in the marrow also) strongly suggests that in the chronic leukæmias it is not only the granulocytic series but the whole myeloid system that is affected and that the differences between cases depend upon the intensity of the proliferation of one or other of the cell series.

Differences between the Myeloid Composition of the Spleen and Bone Marrow
Table 23 presents the splenograms and myelograms of 5 untreated cases in which the results of the sternal punctures have been given in percentages in order to make the values directly comparable. The sternal marrow is generally found to contain more immature myelocytes than the spleen which contains more mature myelocytes and metamyelocytes than the marrow. That is to say the bone marrow in chronic myelocytic leucoses shows a greater degree of shift to the left than does the myeloid tissue of the spleen. The number of myeloblasts in the two organs shows no constant difference and indeed in some cases these cells were rather more numerous than in the spleen. The total number of myelocytes shows variations: sometimes being greater in the marrow sometimes in the spleen while erythroblasts also show no constant relationship.

paramyeloblastic aggravation which will be usually refractory to treatment and will lead to death in a few weeks or months

The structural characters of leukæmic cells have been discussed elsewhere (3) in collaboration with Rohr. In spleen films the myelocytes show the same types of abnormality of structure and development as in the blood and marrow. Thus there is dissociation of maturation of nucleus and cytoplasm and persistence of nucleoli sometimes even when the nucleus has become indented. On the other hand the granules may be very immature and remain clotted together at a time when the nucleus is already mature.

Erythroblasts As shown in the table erythroblasts are always found in spleen films. In myelocytic leucoses polychromatic forms

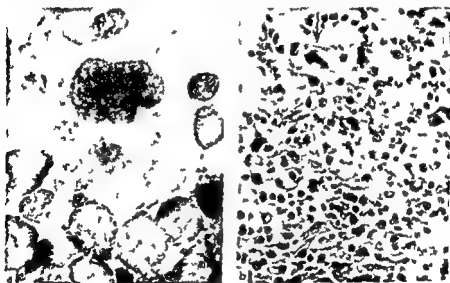


FIG. 46 Megakaryocytes in the spleen in chronic myeloid leucoses
(a) Film (b) Section

are much more numerous than are basophilic and oxyphilic ones while occasionally large basophilic pro erythroblasts are found. Mitoses are commonest among the polychromatic types. Cases in which the erythroblasts are as high as 13 per cent or occasionally 33 per cent (Case 10) are not very uncommon and cells of the erythropoietic series may be more numerous than in marrow films. Such an excess of erythroblasts in spleen puncture is thus a regular feature of myelocytic leucoses but one should not regard a case as being an erythroleukæmia unless the erythroblasts are also greatly increased in the marrow.

Megakaryocytes These cells can be found in the spleen in almost all cases of myelocytic leucosis (Fig 46 a). We were unable to find them in only 2 out of 16 cases. The distribution of the cells in

films ■ irregular perhaps because their large size interferes with their being separated from their connections. If a sufficient amount of material is obtained for the preparation of histological sections it is easy to see how much more numerous these cells are than in the case of films (Fig 46 b). It is as equally true of the sternal marrow in which megakaryocytes are approximately 10 times more numerous in sections than in films (Moeschlin (6)). Such cells may be extremely numerous in the so called megakaryocytic leucoses (see later).

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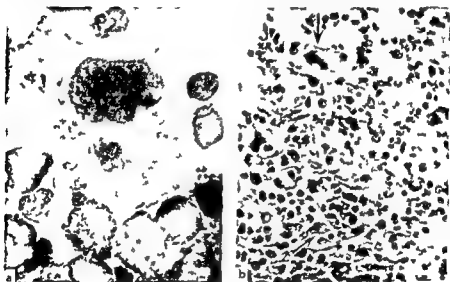


FIG. 46. Megakaryocytes in the spleen in chronic myeloid leucoses
(a) Film (b) Section

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Megakaryocytes. These cells can be found in the spleen in almost all cases of myelocytic leucosis (Fig 46 a). We were unable to find them in only 2 out of 16 cases. The distribution of the cells in

by the spleen such as occurs in other examples of hypersplenism but the presence of numerous mitoses seems incompatible with this. In the present state of our knowledge it is impossible to ascertain what other factors play a part.

Tropeano has recently confirmed our findings. In 10 cases of leucoses (8 chronic and 2 acute) he found that erythropoiesis was more active in the spleen than in the marrow. Also he states that he found no differences in the granulocytes in the two organs. If however one examines his myelograms and splenograms carefully one finds that (except in Cases 6 and 7) there is a larger number of immature forms (myeloblasts and pre-myelocytes) in the marrow than in the spleen. Case 7 is to be excluded because the immature cells were overshadowed by the great proliferation of reticulo-endothelial nature (2.4 per cent). It is also possible that in his Case 6 in which there were more immature cells in the spleen than in the marrow he was really dealing with the terminal stage of a leucosis in which there was a gradual acute dedifferentiation beginning.

Myelograms in Chronic Myeloid Leukæmia
(and mustard gas for further cases)

Granulocytic series												Lymphocytes Total
Myeloblasts	Myelocytes				Neutrophiles			Mature				
	Immature	Half mature	Mature	Metamyelocytes	Total	Staff	Segmented	Total	Eosinophiles	Basophiles	Monocytes	
2.4	11.0	22.0	3.6	14.6	51.2	27.4	10.4	32.8	1.1	4.0	0.5	1.4
	33.0		18.2									
1.5	13.6	30.8	5.0	9.7	59.1	76.9	4.6	31.5	1.4	1.9	0.5	0.4
	44.4		14.7									
1.7	7.0	16.0	11.4	15.7	40.1	23.6	6.6	30.2	0.6	3.3	1.1	8.0
	23.0		27.1									
0.4	9.4	22.6	4.5	9.7	46.2	33.7	13.4	47.1	1.8	1.5	0.4	1.4
	32.0		14.2									
0.5	1.3	3.9	5.5	12.2	27.9	25.1	12.0	37.1	2.2	3.0	0.8	19.7
	5.2		17.7									
1.0	10.4	32.3	6.0	8.0	56.6	23.0	6.8	79.8	3.0	1.2	0.6	1.0
	42.6		14.0									
4.3	6.7	9.3	17.3	12.0	45.3	10.8	6.0	16.8	3.9	8.2	—	8.3
	16.0		29.3									
3.3	5.4	0.3	11.4	10.2	47.3	4.5	13.6	38.1	3.6	5.5	—	1.0
	25.7		21.6									
7.1	3.7	8.5	12.6	6.9	31.7	18.2	11.1	29.3	5.6	9.0	—	7.1
	17.2		19.5									
7.7	4.2	14.5	8.7	5.7	37.6	15.9	7.4	23.3	3.6	2.7	—	9.0
	18.7		13.9									

It was however found that erythroblasts were always more numerous in the spleen than in marrow films This is certainly not due to admixture of blood because in all our cases the spleen juice obtained by puncture was much less bloody than that from the marrow Further, as mentioned below, the shift to the left of the myelogram as compared with the splenogram persists after treatment with arsenic and X rays 12, after the blood picture has more or less returned to normal (Table 27)

Enumeration of the mitoses shows that the excessive number of immature forms in the marrow is accompanied by more numerous cell divisions *There can therefore be no doubt about these differences between the spleen and marrow in the cellular composition*

What may be the Cause of this Difference? The first possibility to occur to one is that there is a hormonal inhibition of the marrow

TABLE 23 *Simultaneous Splenograms and*
(See chapter on urethane arsenic

Case number	A ■ sex		Erythroblasts			
			Basophile	Polychromatic	Orthochromatic	Total
1	64 ♂ (68)	Spleen	0.6	3.1	2.9	6.6
		Marrow	0.6	2.4	0.7	3.7
2	34 ♂ (116)	Spleen	0.8	3.6	0.4	4.8
		Marrow	0.1	1.0	—	1.1
6	34 ♂ (107)	Spleen	1.5	8.7	3.2	13.4
		Marrow	1.0	4.8	0.9	6.7
9	44 ♀ (99)	Spleen	0.8	9.9	2.5	13.2
		Marrow	0.1	0.7	—	0.8
10	33 ♀ (18)	Spleen	10.0	4.8	0.4	15.2
		Marrow	9.5	10.9	1.0	21.4

In only 1 case, which had been treated with urethane we found a larger number of immature cells in the spleen than in the marrow. At this time the blood picture showed no signs of an imminent acute exacerbation but the condition in the spleen suggested that this would occur shortly and in fact it did so 2 weeks later. As Rohr has pointed out such terminal conversion of chronic myeloid leukemia into acute paramyeloblastic leucosis often commences in the spleen at a time when the bone marrow is relatively unaltered. We have however recently seen a case in which the terminal differentiation started in the marrow and not in the spleen. Our present view is that the presence of more immature granulocytes in the spleen than in the marrow is to be regarded as being indicative of an approaching terminal phase.

DIFFERENCES IN THE FREQUENCY AND DISTRIBUTION OF MITOSES IN THE SPLEEN AND BONE MARROW

Only an outline of our observations will be given here as we intend to publish them in detail later. In the spleen, as in the marrow in chronic myelocytic leucosis one finds numerous mitoses in every type of cell from the myeloblasts to the myelocytes and metamyelocytes. In order to obtain a quantitative assessment of the individual stages of mitosis and their distribution among the various types of granulocytes we have performed serial examinations of the sternal marrow and the spleen in 2 untreated cases. The results will be found in Table 24.

We have been able to confirm Fieschi's statement that the percentage values of mitoses in the sternal marrow in chronic myelocytic leukemia are approximately the same as those of mitoses in normal granulocytes in the marrow. Thus Rohr in two normal persons found a mitotic index of 1.8-2.5 per 1 000 whereas in our 2 cases of leukemia the values in the sternal marrow were 3-3.6 per 1 000 respectively. The normal values given by other writers also lie within these limits thus Picena gives 1-2 per 1 000, Fieschi 1 per 1 000 with variations up to 5 or 6 per 1 000 and Weerdts 2.9 per 1 000. *The mitotic index in the marrow in chronic myelocytic leukemia is thus only slightly above normal i.e. the relative number of mitoses is only slightly increased but naturally the total number is greatly raised because of the enormous extension of active leukæmic marrow tissue.*

The state of granulocyte mitoses in the spleen as compared with that in the sternal marrow is very surprising. Thus in both our cases there were strikingly fewer mitoses in the spleen than in the marrow. Marrow films on the average show a three or four times higher mitotic index than do spleen films. At present we can scarcely do more than guess at the significance of these findings. Neither of our cases had received any treatment so that no external factor

doses (2 500-3 000 r) complete aplasia of the marrow occurred which however was followed by slight regeneration

The literature does not contain any detailed account of the effect of X irradiation of the bone marrow in chronic myelocytic leucoses (except 1 case recorded by Stephens) or on the extramedullary myeloid tissue of the spleen. In 1 case we punctured the spleen 24 hours after 120 r had been given and found striking fragility of the cells together with many degenerate myelocytes some of which showed nuclear pyknosis and formation of vacuoles in the cytoplasm. *There were many macrophages but the erythroblasts showed no structural changes and seemed to be slightly more numerous than in the previous puncture.* The fragility of the cells was so great that a detailed qualitative assessment was impossible and for the same reason it was impossible to judge of the frequency of mitoses. In the following case the changes in the spleen and marrow were

TABLE 25

	Before irradiation			Four months after irradiation		
	Hæmo-gram	Spleno-gram	Myelo-gram	Hæmo-gram	Spleno-gram	Myelo-gram
Total leucocytes (in 1 000 s)	144.0	—	—	15.5	—	—
Myeloblasts	—	—	—	—	—	—
Myelocytes immature	—	—	—	—	—	—
Myelocytes half mature	—	—	—	—	—	—
Myelocytes mature	—	—	—	—	—	—
Metamyelocytes	—	—	—	—	—	—
Total myelocytes	15	—	—	4½	—	—
Neutrophiles staff	55	—	—	45½	—	—
Neutrophiles segmented	21	—	—	34½	—	—
Eosinophiles (myelocytes and mature)	1	—	—	—	—	—
Basophiles (myelocytes and mature)	6½	—	—	6½	—	—
Monocytes	1	—	—	5½	—	—
Lymphocytes	½	—	—	3½	—	—
Macrophages	—	—	—	—	+	—
Plasmacyt retic. cells	—	0.3	—	—	0.3	—
Pulp cells	—	—	—	—	0.1	—
Erythroblasts basophile	—	0.3	1.0	—	0.3	4.0
Erythroblasts polychrom	—	4.5	1.3	—	0.8	10.7
Erythroblasts orthochrom	—	2.6	1.0	—	—	0.9
Erythroblasts total	—	7.4	3.3	—	1.1	15.6
Myeloblasts	—	0.4	0.4	—	—	0.3
Myelocytes immature	—	4.1	4.2	—	1.0	6.6
Myelocytes half mature	—	7.3	13.0	—	2.8	10.9
Myelocytes mature	—	11.0	13.2	—	7.3	14.6
Metamyelocytes	—	15.2	7.3	—	2.3	14.2
Total myelocytes	—	37.6	37.7	—	8.4	46.3
Neutrophiles staff	—	29.6	40.1	—	16.0	6.5
Neutrophiles segmented	—	8.8	11.2	—	44.9	6.8
Eosinophiles mature	—	1.7	4.3	—	0.2	1.5
Basophiles mature	—	2.6	1.9	—	6.0	0.9
Monocytes	—	0.1	0.1	—	4.0	0.3
Total lymphocytes	—	11.5	1.0	—	18.9	1.8
Megakaryocytes	—	+	+	—	+	+

can be responsible for the appearances. Even in the cases discussed below the mitotic index in the sternal marrow was three times higher than that in the spleen. As far as one may base conclusions on only 6 cases this would seem to be an invariable difference between the myeloid tissue of the spleen and marrow in chronic myeloid leukæmias. It is certain that the reduction of the mitotic index is not due to admixture of blood with the spleen juice because none of our films contained much blood. The speed of mitoses in myelocytes in the marrow and spleen is not likely to be different but it is conceivable that there might be a hormonal inhibition of mitosis in the marrow which would prolong it and thus account for the greater number of cell divisions, but at present little is known about the reciprocal relations of the spleen and marrow. Splenectomy in leukæmias appears to exert an unfavourable influence in chronic myelocytic leukæmia (Forkner) leading to rapid deterioration.

If the percentage distribution of cells in marrow and spleen films is compared one finds distinctly more mature forms in the latter (Table 23). This seems to indicate that there is some kind of hormonal influence in the sense of the increase of immature forms in the marrow and possibility of delay in the maturation of myeloid cells. If this were so an increased number of mitoses in the marrow might well be the result of the presence of numerous primitive cells, which in the spleen also show more mitoses than do the older types. This explanation is admittedly not entirely satisfactory.

If we examine the distribution of mitoses in the various stages of cell development we find that in the spleen the mitoses are fairly evenly distributed between the immature and the half mature myelocytes 2-3 per 1 000 whereas in spite of their great number mitoses in the more mature cells (mature myelocytes and meta myelocytes) are scantier (Table 24). *In chronic myelocytic leucoses therefore the myelocytes are to be regarded as maturing elements while new formation of daughter cells occurs by mitotic division of the immature and half mature forms.* This formative activity of the immature forms is even more noticeable in the sternal marrow. Thus in Case 2 6 per 1 000 of the immature myelocytes and 5 per 1 000 of the half mature ones were in mitosis (Table 24).

THE EFFECTS OF X RAYS ON THE SPLEEN AND MARROW

Rachmilewitz (1947) who investigated the radio sensitivity of cultures of normal marrow cells observed decrease in the number of myelocytes and a greater sensitivity of the more mature forms of both granulocytes and erythroblasts. Denstad observed decrease of red and white cells after irradiation of the sternum with 300 r but even then the erythroblasts were rather more sensitive. With larger

examined before X ray treatment and 4 months after it, the total doses given over the splenic area being 800 r. The total number of leucocytes corresponding to 20 mitoses was counted on both occasions (Table 25 and Fig 46 c)

This example demonstrates that the therapeutic effect of X rays does not depend upon a general influence but purely upon the action of irradiation on the myeloid tissue in the treated area. Rays cause three types of changes in the affected myeloid tissue. (1) A distinct reduction in the amount of myeloid tissue in the spleen, this is both absolute as shown by decrease in size of the organ and also relative inasmuch as there is a percentage decrease of granulocytes and in consequence, a relative rise of lymphocytes. (2) There is a greater reduction of the most immature types associated with a relative increase of the mature ones; this may well be the most important therapeutic effect of the treatment. In this case spleen puncture before irradiation showed 38 per cent of immature cells but only 8 per cent after treatment. This is probably attributable to a greater degree of sensitivity of the more immature cells. Certainly it is improbable that irradiation causes accelerated maturation as Isaacs thought. The number of erythroblasts also falls after irradiation. (3) In the directly irradiated area there is an absolute and relative decrease of mitoses probably as a result of destruction of the most immature elements. Thus before irradiation the mitotic index in the spleen was 1.0 per 1,000 and after the irradiation 0.1 per 1,000, i.e. a decrease to a tenth part of the original value (Table 26 and Fig 46 c).

It is striking that the sternal marrow and also the remainder of the bone marrow except that in the area of irradiation still show typical leukaemic changes with excess of immature forms and many mitoses even after a complete course of radio therapy, in fact the typical changes may become more marked. A comparison of the blood picture with the myelogram shows that the increase in the number of mitoses in the marrow cannot be attributed to diminished admixture of granulocytes from the blood. If such increase of mitoses in the bone marrow after irradiation of the spleen can be confirmed in other cases of chronic myelocytic leucosis we should have a reasonable explanation of the decrease of hormonal inhibition of the marrow which follows diminution in size of the spleen. The only observation which we are aware is that of Stephens who observed 1 case in which 500 r were given over the thorax and spleen 5 months earlier. He found that in the sternal marrow immature myeloid cells had fallen from 52.7 to 24.3 per cent. Biopsy from the sternum before irradiation of the thorax showed increased hyperplasia of the myeloid tissue whereas 5 months later the marrow was poor in cells and fibrotic. This case is not directly comparable with ours because the sternal marrow as well as the

TABLE 26

	Spleen							Marrow								
	Prophase	Metaphase	Anaphase	Diastere	Dispersed	Total cells in mitoses	Number of cells per 1 mitoses	Mitoses per 1 000	Prophase	Metaphase	Anaphase	Diastere	Dispersed	Total cells in mitoses	Number of cells per 1 mitoses	Mitoses per 1 000
(a) Before irradiation																
Myeloblasts	—	—	—	—	—	0	103	0	—	—	—	—	—	0	69	0
Myelocytes immature	—	—	—	—	—	2	1 063	1.8	—	—	—	—	—	3	1 175	4
Myelocytes half mature	2	5	1	—	—	8	1 893	4.2	1	14	1	2	1	18	2 346	7.7
Myelocytes mature and meta	—	6	3	—	—	10	6 796	1.4	—	13	—	—	—	17	3 699	4.6
Mature granulocytes	1	—	—	—	—	0	11 104	0	2	—	—	—	—	0	10 035	0
Total	4	12	4	0	0	20	20 959	0.95	5	30	3	2	2	40	17 374	2.3
Cells per 100 mitoses	20	60	20	0	0	100	104.795		12.5	75	7.5	5	5	100	43.310	
Mitotic index																
(b) After irradiation																
Myeloblasts	—	—	—	—	—	0	0	0	—	—	—	—	—	0	35	0
Myelocytes immature	—	—	—	—	—	0	763	0	—	7	—	—	—	9	973	9.7
Myelocytes half mature	1	1	—	—	—	2	738	2.7	4	8	4	—	—	16	1 524	10.5
Myelocytes mature and meta	—	—	—	—	—	0	1 212	0	3	9	3	—	—	15	4 076	3.7
Mature granulocytes	—	—	—	—	—	0	18 746	0	—	—	—	—	—	0	5 096	0
Total	1	1	0	0	0	2	20 959		7	24	9	0	0	40	11 604	
Cells per 100 mitoses	50	50	0	0	0	100	1 047.950	0.09	17.5	60	22.5	0	0	100	29.010	3.4
Mitotic index																

examined before X ray treatment and 4 months after it, the total doses given over the splenic area being 800 r. The total number of leucocytes corresponding to 20 mitoses was counted on both occasions (Table 25 and Fig 46 c)

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Number and Distribution of Mitoses in Spleen and Sternal Puncture Before and After λ Irradiation

	Spleen						Marrow					
	Prophase	Metaphase	Dispersed	Total cells in mitoses	Number of cells per 1 mitoses	Mitoses per 1 000	Prophase	Metaphase	Dispersed	Total cells in mitoses	Number of cells per 1 mitoses	Mitoses per 1 000
(a) Before radiation												
Myeloblasts	—	—	—	0	103	0	—	—	—	0	69	0
Myelocytes immature	—	—	—	2	1 063	18	—	—	—	5	1 175	4
Myelocytes half mature	2	5	1	8	1 893	47	1	1	1	18	2 346	77
Myelocytes mature and meta	—	6	3	10	6 796	14	2	13	1	17	5 699	46
Mature granulocytes	1	—	—	0	11 104	0	2	—	—	0	10 035	0
Total	4	17	4	0	20 959	0.95	5	30	3	40	17 374	23
Cells per 100 mitoses	0	60	0	100	104 795		12.5	75	7.5	100	43 310	
Mitotic index												
(b) After irradiation												
Myeloblasts	—	—	—	0	0	0	—	—	—	0	35	0
Myelocytes immature	—	—	—	0	263	0	—	7	—	9	923	97
Myelocytes half mature	1	1	—	2	738	27	4	8	4	16	1 524	105
Myelocytes mature and meta	—	—	—	0	1 212	0	3	9	3	15	4 026	37
Mature granulocytes	—	—	—	0	18 746	0	—	—	—	0	5 096	0
Total	1	1	0	2	20 959	0.09	7	24	9	40	11 604	34
Cells per 100 mitoses	50	50	0	100	1 047 950		17.5	60	22.5	100	29 010	
Mitotic index												

inhibition as a result of arsenic Erythropoiesis in the spleen shows distinct increase as a result of arsenic and in our second case (Table 27) it increased by 5 times within 3 weeks

TABLE 27

Spleen and Marrow before and after Arsenic Treatment

		Case 6 (34 male)				Case 14 (54 male)			
		Before arsenic		After 16 days arsenic (Fowler)		Before arsenic		After 24 days arsenic (Fowler)	
		Sternum	Spleen	Sternum	Spleen	Sternum	Spleen	Sternum	Spleen
100°	Macrophages	—	01	—	+	—	02	02	02
	Plasmacyt retic cells	—	03	01	11	—	01	02	26
	Pulp cells	—	—	—	01	—	—	—	06
	Erythroblasts basophile	10	15	77	29	08	32	20	38
	Erythroblasts polychromatic	48	87	132	117	15	58	186	276
	Erythroblasts oxyphile	09	32	07	12	—	16	72	162
	Erythroblasts total	67	134	216	158	23	106	278	476
	Myeloblasts	10	05	03	02	59	17	20	02
	Myelocytes immature	104	13	27	10	76	68	24	07
	Myelocytes half mature	322	39	113	26	156	134	52	28
	Myelocytes mature	60	55	59	34	26	131	22	50
	Metamyelocytes	80	122	71	39	62	93	54	36
	Total myelocytes	566	229	270	109	320	443	152	116
	Neutrophiles staff	230	251	302	121	416	187	304	107
	Neutrophiles segmented	68	120	125	149	74	103	92	54
	Eosinophiles mature	30	22	48	63	39	22	84	57
	Basophiles mature	12	30	12	47	51	50	42	08
	Monocytes	06	08	09	24	04	06	—	—
	Total lymphocytes	10	197	14	315	14	80	24	156
	Megakaryocytes	01	+	+	—	+	—	+	—
<i>Hæmogram</i>									
100	Total leucocytes	81 200		11 500		179 000			
	Myeloblasts	—		—		5			
	Myelocytes immature	4½		—		2			
	Myelocytes half mature	2½		1		10			
	Myelocytes mature	5		—		2½			
	Metamyelocytes	4		1½		5			
	Total myelocytes	16		2½		19½			
	Neutrophiles staff	41		0		44			
	Neutrophiles segmented	31½		48½		13			
	Eosinophiles (myelos and mature)	1½		7½		3½			
100	Basophiles	3½		3½		7½			
	Monocytes	2½		6½		2½			
	Lymphocytes	4		11½		5			

In the sternal marrow we were able to confirm the observations of Stephens that the changes are similar to those in the spleen although they are more striking on account of the initially more immature picture. Thus in the first case the number of myelocytes fell to half while the mature granulocytes increased by a third and

erythroblasts to treble the initial number. Case 14 (Table 27) showed a smaller decrease of immature granulocytes to one third of their initial number with almost complete disappearance of myeloblasts and immature myelocytes. Within 3 weeks the erythroblasts had increased to eleven times their initial number.

In Case 8 the splenogram which is shown in Table 22 cannot be amplified by myelograms because we obtained no good films. This patient had been taking small doses of arsenic (8-12 drops of Fowler's solution twice a day) for 8 years and was continuously fit for work. Initially the leucocyte count was in the region of 195 000 but for a number of years it remained between 8 000 and 12 000 with only 1 or 2 per cent of immature cells in the blood films. Even the shortest interruption of the arsenic was immediately followed by a rise of granulocytes. After 5 years' continuous treatment with arsenic the splenogram showed a decrease of myelocytes to one quarter of its former value while immature forms were also greatly reduced (Table 22). Case 8 the erythroblasts having quadrupled themselves and the lymphocytes having doubled themselves.

Thus treatment with arsenic results in a distinct percentage decrease of granulocytes particularly of the immature myelocytes both in the spleen and the marrow while there is a relative increase of lymphocytes in the former and erythroblasts in the marrow and sometimes in the spleen (Fig. 46 d).

Effects on mitoses. The far reaching therapeutic effect of arsenic on the leukæmic process is certainly not fully explained by the marrow although there is definite decrease of granulocytes in the spleen and marrow. According to our observations (Moeschlin (19)) arsenic is still the most powerful therapeutic agent in chronic

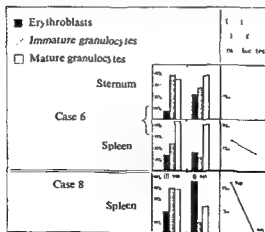


FIG. 46 d. Sternal and spleen films before and after arsenic treatment (For explanation see text)

myelocytic leucoses and is to be preferred to X rays or urethane for continuous treatment because it has also a beneficial effect on the expectation of life as Forkner has also stressed. This treatment does not however have any effect on the terminal paramyeloblastic phases of chronic myelocytic leukæmias and, indeed, the patient just mentioned died of such an attack after 9 years of treatment.

We have investigated the effect of arsenic on the number of mitoses in both the above cases because in view of Dustin's work it seems reasonable to assume that arsenic in the leukæmias acts as a mitotic poison. Our results are shown in Table 28.

The result of these investigations clearly shows that arsenic in therapeutic doses produces a well marked decrease in the number of mitoses in granulocytic tissues. After 2 weeks' treatment with arsenic the number of mitoses in the spleen fell to approximately one half and in the marrow to approximately one third of the values found before treatment commenced (Fig. 46 d).

Such inhibition of mitosis persists during continuous treatment with doses of arsenic that are just sufficient to maintain the leucocytes at numbers slightly above normal. The mitoses fall to very low values as is shown in Case 8 —

<i>Before arsenic treatment</i>	<i>After 5 years' continuous arsenic treatment</i>
40 mitoses in 15 580 granulocytes	No mitoses in 15 580

In this case therefore the number of mitoses has decreased by at least 40 times as a result of persistent treatment with arsenic.

The therapeutic effect of arsenic in the leukæmias therefore depends mainly upon the toxic inhibition of mitosis. Further in analogy with the observations of Rohr on the effect of arsenic on erythropoiesis in pernicious anaemia there is possibly a beneficial effect on cellular maturation. Certainly one of the greatest advantages of arsenic is the fact that it can be administered in therapeutic doses for years on end without damaging the formation of red corpuscles (Table 22 Case 8).

During arsenic treatment of myeloid leukæmias there is a shift to the left of the mitotic curve due to an increase in the number of prophases viz. in the spleen from 5 per cent to 20 per cent and in the sternal marrow from 13 per cent to 25 per cent. Fieschi asserts that this increase in the number of prophases depends upon the stimulation of karyokinesis. This is certainly not true of the mitotic curve of chronic leukæmia treated with arsenic because almost certainly there is a toxic delay in the course of prophase. It is not possible to obtain any definite information as to the duration of mitosis by simple enumeration of the various stages and the

conclusions of Fieschi and Kienle, who attribute the gradual shift of the mitotic curve to acceleration or retardation of the various stages of cell division, are purely hypothetical.

THE EFFECTS OF URETHANE ON THE SPLEEN AND MARROW PICTURES IN THE LEUCOSIS

In view of the observations of Haddow, Sexton and Pitterson on inhibition of tumour growth and its value in the treatment of chronic leucoses it seems useful to discuss the haematological changes that occur in the spleen and marrow after urethane.

A brief description of the effects of urethane on the peripheral blood will be given as an introduction.

Moeschlin (18) and Meili have shown that the usual therapeutic dose of urethane (3 g daily) causes a fall of lymphocytes in most normal people. Indeed, a genuine lymphocytosis may develop. The granulocytes fall slightly after a preliminary rise, but in specially susceptible persons and in the presence of previous marrow damage, severe granulocytopenia may develop. As a rule neither erythropoiesis nor thrombocytopoiesis is affected but, again, in susceptible persons, there may be decrease. As Table 29a shows, there is no diminution of mitoses even after 4 weeks on urethane.

TABLE 29a

*Mitoses (Mitotic Index) before and after Urethane
(Healthy Subjects)*

	Case number	Before urethane	After urethane
Lymphoblasts	1	14 ⁰ / ₁₀₀	32nd day 17 /
	2	14 /	13th day 16 / 27th day 14 /
	3	14 /	14th day 16 /
Granulocytes	1	13 ⁰ / ₁₀₀	32nd day 0 /
	2	0.9%	13th day 0 / 27th day 1.2 /
	3	1.3 /	14th day 0.8 /

A functional agranulocytosis without granulocytopenia can be produced by urethane in the normal individual as Sindkuhler (1949) has demonstrated. The greatly lowered resistance against infections of cases undergoing urethane treatment can be explained by the fact that in healthy persons the application of daily 2 gr urethane lowers the phagocytic index from 80 to 50 per cent.

In animal experiments (Moeschlin (20) and Naef) there was a distinct susceptibility of lymphatic tissue, granulocytes and red

TABLE 29b
Myelogram and Splenogram Chronic Lymphatic Leukemia before and after Urethane

	A A, aged 33 male Myelogram			Splenogram 113th day	D L, aged 59 female Myelogram			Splenogram		
	1st day	27th day	113th day		1st day	7th day	93rd day	1st day	93rd day	
Erythroblasts	32	22	290	34	46	42	30	—	0+	
100 { Immature granulocytes Mature granulocytes Lymphocyte	50 54 89.6	12 40 94.8	168 74 75.8	30 54 87.5	17 10 97.8	06 34 96.0	10 27 96.8	01 16 98.2	01 64 93.3	
<i>Hemo gram</i> Total leukocytes Immature granulocytes Mature granulocytes Lymphocytes Thrombocytes	19 000 5 28 67 8 000	5 000 — 46 54 63 000	5 600 3 39 58	5 600 3 39 58	81 00 — 65 93.5 66 000	46 350 — 7 92.5 16 000	17 200 — 32.5 66.5	81 200 — 65 93.5 66 000	17 700 — 32.5 66.5	

cells being affected later. The only exception was the cat, in which we found that the granulocytic system was as sensitive as that of patients with chronic myelocytic leucosis. Quite moderate doses of urethane can produce fatal panmyelophthisis in cats (Moeschlin (20, 26) and Bodmer). Clearly, then, there is great variability in the susceptibility of different blood cells and also of different animals.

(a) CHRONIC LYMPHATIC LEUCOSES

The therapeutic effect of urethane is usually less than in chronic myelocytic leucoses (for literature see Moeschlin (19, 21)). A slight reduction of lymphocytes is found in spleen and marrow punctures (Table 29b).

The great reduction of lymphocytes that may occur after a treatment of 1 month or longer in the peripheral blood before there is any or only little change in the cellular composition of the spleen and marrow shows that the main action of urethane is on cell proliferation as well as in reducing the amount of lymphatic tissue.

Unfortunately in the 1st and 3rd cases no spleen puncture was carried out before treatment (because of an hæmorrhagic diathesis) but even so there was a demonstrable increase of extramedullary blood formation in the spleen (up to 6 per cent), presumably as a result of depression of lymphocytopoiesis.

In fact after urethane the lymphocytes in spleen puncture may fall to 85-89 per cent unlike untreated cases in which they are usually above 95 per cent. No determination of the mitotic index was possible because of the rarity of cell division in lymphocytes (see p. 40).

We would stress the inadvisability of giving urethane in any case of aleukæmic lymphatic leukæmia in which there is leucopenia because there is usually very extensive infiltration of the marrow with lymphocytes. In one such case urethane caused complete failure of the already inadequate marrow.

(b) CHRONIC MYELOCYTIC LEUCOSIS

As in chronic lymphatic leukæmia urethane seems to have a slightly stimulating effect before its depressant one manifests itself as is shown by a slight increase of cells in the peripheral blood during the first few days of treatment (Moeschlin (17) Storti (3)). It seems probable therefore that the action of urethane is on cell division. Within 7-14 days varying with the dose and the individual the granulocytes begin to fall (in lymphatic leucoses the decrease may commence much later e.g. 3-4 weeks).

Is this decrease brought about by destruction of the leukæmic cells? If this were so the blood uric acid and the excretion of uric acid would arise as it does after radio therapy but we have not found this in any of our urethane cases. Clearly, then, the drug

TABLE 29C
Mitotic Index in Spleen and marrow Punctures in Chronic Myeloid Leukemia before and after Urethane Treatment

	Case 1 (aged ♀ female)						Case 2 (aged 66 mal)						Case 3 (aged 3 male)					
	Mitotic index per 1000						Mitotic index per 1000						Mitotic index per 1000					
	Spl en		Marrow				Spleen		Marrow				Spleen		Marrow			
	1st day	35th day	1st day	35th day	1st day	35th day	1st day	38th day	1st day	38th day	1st day	32nd day	1st day	32nd day	1st day	32nd day	1st day	32nd day
Erythroblasts	89 /	0 /			62 /	59 /	1 /	145 /	117 /	85 /					17 / ₁₀₀	163 / ₁₀₀		
Myceloblasts Immature myelocytes Half mature myelocytes Mature myelocytes Mature granulocytes	4 /	14 /			315 / ₁₀₀	79 /	4 / ₁₀₀	603 / ₁₀₀	04 /	05 /					019 / ₁₀₀	62 / ₁₀₀		

must act by inhibiting the formation of leukæmic cells. Then again there are no distinctive structural changes suggestive of cell destruction (Storti (3) Moeschlin (17)), although the cytoplasm of some of the younger cells may be vacuolated. This change also occurs in experimental animals (Moeschlin (20) Tischendorf) but is much less striking than the other phenomena. Bock has also described clumping of the peroxidase granules which may lie at the edge of the cell. Storti tells me that he also has observed this.

Changes in Spleen Puncture Table 29c shows the findings in 3 cases in each of which 50 000 cells were examined in spleen and marrow films in order to enumerate the mitoses. (I am deeply grateful to the Misses Ernst and Obrecht for this time consuming labour.)

In the first case after 35 days on urethane the blood picture had become much more normal but apart from a distinct increase of erythropoiesis the proportions of granulocytes showed little change. Apparently there had been considerable retrogression of leucopoietic marrow and increase of erythropoietic tissue although the number of immature white cells relative to the mature forms showed little change only the segmented neutrophils had increased. In this case the spleen only became 3 cm. smaller in 35 days.

How can the improvement in the blood picture be explained (the leucocytes fell from 194,000 to 25 000)? It is not really conceivable that the very moderate retrogression of the leucopoietic tissue in the spleen and marrow could be the cause of so great a metamorphosis: there must have been the additional factor of inhibition of cell division. The view is confirmed by our observations on mitotic activity. Table 29c shows the number of mitoses per 50 000 cells. Fig. 47 makes clear that the granulocyte mitoses in the spleen (after 35 days on urethane) fell to a quarter of the initial number while those of the erythroblasts were doubled.

In the above case the improvement following urethane was due to a selective inhibition of granulocyte proliferation. In the other 2 cases in which the number of mitoses was counted (Table 29c) there was a similar but less intense fall of the mitotic index in spleen films. Storti (3) and Mauri as well as Schulze and Bock have confirmed our findings.

Sternal Puncture As a rule there is a distinct rise in the mitotic index of the erythropoietic cells but rather surprisingly little change in that of the granulocytes. Indeed in Case 3 there was increase in the number of granulocyte mitoses. Until further investigations have been carried out there can be no explanation of this peculiar difference between the conditions in the spleen and marrow. The condition is however comparable with that following X irradiation of the spleen in which diminution in the size of the organ perhaps decreases the intensity of the splenogenic inhibition of the marrow.

with a consequent rise of the mitotic index (p 144) The failure of urethane as an inhibitor of the marrow only is the more surprising because arsenic affects spleen and marrow equally

We have already shown that urethane has practically no inhibitory effect on myelopoiesis in the normal subject and it may be that the different response of the spleen and marrow in chronic leukaemias is due to a different mode of granulocytopoiesis in the two organs Thus it might be that only the spleen has undergone a

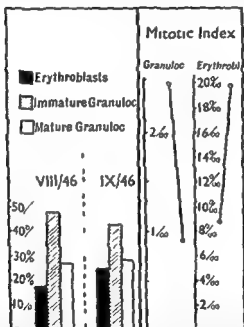


FIG 47 a Effect of urethane on mitoses in the spleen in chronic myeloid leukaemia After 35 days treatment with urethane (99 g) there is an increase of erythroblasts but the proportion of myeloid leucocytes shows little change. There is however decrease of the mitotic index of the granulocytes to one third of their original number i.e. to the number of mitoses per 1 000 immature cells. The mitotic index of the erythroblasts has risen to double the original value. The main effect of urethane is thus a selective inhibition of mitotic activity in the abnormal leukaemic white cells

leukaemic change and is therefore more sensitive to urethane. The hypothesis is attractive but lacks evidence. Thus splenectomy usually has a bad effect on chronic myelocytic leukaemia (Forkner Gasser *et al*) while as we have already pointed out the granulocytes of the marrow show also structural leukaemic abnormalities. Clearly then this series of cells is atypical in both organs at least in the advanced stages of the disease. Then again we have seen a terminal paramyeloblastic exacerbation commence in the marrow and not in the spleen (Case 2 p 160). We are therefore forced to accept the view that the apparent paradox is perhaps the result of

decreased inhibition of the marrow, as a result of decrease in the size of the spleen

Care is needed in the interpretation of mitotic indices. Thus in tissue culture, Bucher observed differences in mitotic activity due to varying concentrations and duration of action of the chemical substances he tested. It is therefore possible although not probable, that the explanation is that there are different concentrations of urethane in the spleen and marrow. It is also possible that the marrow cells but not those of the spleen are regulated by nervous activity. The field thus opened for hematological research is both extensive and important.

We have purposely devoted space to Case 1 because the failure of the marrow and spleen cells to show signs of increased maturity demonstrates that the striking therapeutic effect of urethane cannot be explained by simple depression of the immature cells. Admittedly in our other cases there was extensive metamorphosis of the cellular composition of the spleen and marrow, inasmuch as the immature cells decreased and there was a relative increase of more mature forms. This might occur within 2 or 3 weeks but usually within 3-6 weeks. This change is well shown in Table 29d (Case 3). Here the granulocytes in the spleen fell from 58 per cent to the very low figure of 0.7 per cent within 4 weeks while the lymphocytes rose from 12 per cent to become the predominant cells (67 per cent). At the same time the immature white cells fell to a quarter of their initial value and erythropoiesis became more active than normal. In such cases the reversion to normal of the blood picture can be explained, even in the absence of any special interference with mitosis. There is however inhibition of cell proliferation which affects the younger cells more than the less sensitive mitoses of the more differentiated cells (mature myelocytes). *At present such a selective action of urethane on the young leukæmic cells in the sense of inhibition of the cell proliferation seems to be the best explanation of the effects of urethane.* Indeed as has been shown in animal experiments such damage of immature cells may even lead as far as to produce neoplastic degeneration (Henshaw, Nettleship, Guyer, Jaffe, Moeschlin (20-21) and Naef).

(c) STRUCTURAL CHANGES IN MITOSES

Not only does urethane cause a decrease in the number of mitoses but abnormal forms occur. For instance in spleen films we have observed swollen chromosomes, adhesion of pairs of chromosomes and displaced ones. Bock, Schulze and Storti have reported similar appearances in the marrow while Bastrup Madsen has seen them in tissue cultures of fibroblasts.

Moeschlin (26) and Bodmer found identical structural changes in cats in which panmyelophthisis had been caused by urethane.

TABLE 29d

Myelograms and Splenograms in Chronic Myeloid Leucoses (before and after Treatment with Urethane)

	Case 1 R H (aged 7 female)				Case 2 A E (aged 64 male)				Case 3 P E (aged 3 male)			
	Splenium		Spleen		Spleen		Spleen		Splenium		Spleen	
	1st d y	35th d y	1st day	35th day	1st d y	39th day	39th day	1st day	1st day	3 nd day	1st day	3 nd day
Erythroblasts	58	370	20	327	6	190	71	44	33	594	100	176
Myeloblasts	44	10	41	—	10	08	—	—	52	19	117	—
Myelocyte total	44	476	543	578	510	414	362	104	369	94	460	07
Myelocyte in mature	282	98	301	404	370	356	220	113	773	72	190	—
Myelocytes mature	60	86	96	32	140	58	14	51	96	2	270	07
Neutrophils segmented	320	258	20	169	6	294	174	88	37	80	37	73
Neutrophils segmented	112	474	73	200	114	376	324	444	291	72	14	106
Eosinophiles	10	04	10	—	56	17	09	27	145	95	76	53
Basophiles	44	02	37	01	38	0	96	115	81	36	03	—
Mono cytes	—	—	—	01	—	—	—	10	—	—	—	—
Lymphocytes	8	34	76	42	10	10	06	42	25	09	13	636
Leucocytes	193 600	25 400	193 600	25 400	74 100	15 900	74 100	15 900	111 400	9 900	111 400	9 900
Myeloblast	4	25	4	25	51	—	51	—	16	2	16	—
Myelocyte total	50	32	65	37	51	—	51	—	11	—	11	—
Myelocytes in mature	105	15	105	125	51	—	51	—	5	—	5	—
Neutrophils segmented	31	15	33	15	41	665	242	665	14	57	14	57
Neutrophils segmented	6	47	6	47	50	55	50	55	13	53	13	53
Eosinophiles	05	05	05	05	11	45	11	45	21	1	21	1
Basophiles	1	15	3	15	11	05	11	05	25	—	25	—
Mono cytes	—	—	—	—	11	05	11	05	25	—	25	—
Lymphocytes	35	35	35	35	41	245	41	245	40	7	40	7
Thrombocytes	153 000	107 500	153 000	107 500	540 000	—	540 000	—	746 400	—	746 400	—

decreased inhibition of the marrow, as a result of decrease in the size of the spleen

Care is needed in the interpretation of mitotic indices. Thus in tissue culture Bucher observed differences in mitotic activity due to varying concentrations and duration of action of the chemical substances he tested. It is therefore possible although not probable that the explanation is that there are different concentrations of urethane in the spleen and marrow. It is also possible that the marrow cells but not those of the spleen, are regulated by nervous activity. The field thus opened for hæmatological research is both extensive and important.

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Moeschlin (26) and Bodmer found identical structural changes in cats in which panmyelophthisis had been caused by urethane.

Obviously then urethane has an effect direct or indirect, on the course of cell division a matter we shall return to at the end of the present section

Enumeration of the different phases of mitosis revealed only a

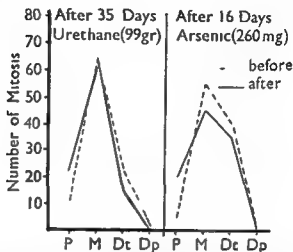


FIG 47 b Distribution of the various stages of mitosis in chronic myeloid leukaemia before and after treatment (spleen puncture) P = prophases M = monaster Dt = diaster Dp = Dispireme No striking change in the mitotic curve can be detected but there is slight increase in the number of prophases This is however within the limits of experimental error

slight increase of prophases (Fig 47 b) so that it is clear that urethane does not act only on one stage of cell division but on the whole karyokinetic process (Moeschlin (17) Schulze Storti (3))

(d) PROLONGED TREATMENT WITH URETHANE

Table 29c shows splenograms and myelograms from 2 cases of chronic myelocytic leucosis which were continuously treated with urethane

We could follow the first case for 3 years From 1945 until April 1946 the patient was treated with arsenic by mouth and was then given urethane At first 4 g were given daily but the dose was gradually reduced to between 0.5 and 1.5 g daily according to the state of the blood picture and during the whole period the patient was fit for work In 18 months the total amount of urethane was 800 g Every attempt to stop urethane was followed by rapid increase of leucocytes and deterioration of the general condition In the spring of 1948 there was a sudden fall of the white cells (to 7 000) and for the first time severe anaemia (56 per cent) In spite of stopping urethane and giving transfusions and penicillin the patient died with a severe pancytopenia the granulocytes having fallen to 350 and the thrombocytes to 5 000 before death Puncture of the spleen and marrow excluded the possibility of a terminal paramyeloblastic exacerbation

The damage of which there were no premonitory signs was

enlargement of the spleen and lymph glands it is not always true. There seems to be aggressive forms of paramyeloblastic leucosis discharging the cells directly from the marrow into the blood stream.

(e) ACUTE LEUCOSIS

Paramyeloblastic leucoses may run a fairly chronic course even without treatment but even so there is some evidence that in some cases urethane has a depressant effect on the abnormal cells in the blood (Moeschlin (19)). We have never been able to observe a revival of myelopoiesis such as Schwarz-Tien and Heilmeyer described each in one special case; indeed in spite of urethane all our cases ran the usual fatal course. Once we had a case in which the spleen and sternal punctures showed almost total disappearance of abnormal cells from the blood, spleen and marrow and a further oddity was that the acute leukaemia had developed in a case of congenital hemolytic jaundice. We have now seen this combination three times (Moeschlin (3)).

Sch. H. aged 26 book keeper

One sister developed hemolytic anemia in 1933. The patient had a first attack of the same condition at the age of 12 (1913) and had had an enlarged spleen ever since.

In March 1937 he was getting paler while the spleen was noticed to be increasing in size (Hb 30 per cent).

On admission to the Clinic the general condition was poor. The spleen was enormous (31 x 21 cm) firm and tender. The liver was enlarged (17 cm in the nipple line) and firmer than normal. Haemoglobin 20 per cent, red corpuscles 1.18 million, reticulocytes 7.8 per cent, leucocytes 102 000—851 per cent, oxydase positive micromyeloblasts containing azure granules and 4 per cent myelocytes. Thrombocytes 38 000. Some spherocytosis. Diminished osmotic resistance of red corpuscles 0.66–0.30. BSR 85, bilirubin 3.5 mg per cent, serum iron 270 gamma, prothrombin 30 per cent.

Course. The leucocytes fell to almost normal numbers after treatment with arsenic but the composition of the white-cell picture did not become less abnormal. Repeated transfusions raised the haemoglobin to 60 per cent. The results of spleen and marrow punctures are shown in Table 29 f.

In spite of continued administration of arsenic (8 drops i.d.s.) there was sudden exacerbation at the end of 3 months, the leucocytes having risen to 169 000 (all micromyeloblasts). When this relapse occurred urethane was started (4 g daily) and within 3 weeks the leucocytes had fallen to 1 500. Urethane was stopped after 87 g had been given but there was no reappearance of normal cells. Spleen and marrow punctures now showed only reticulum cells and a few isolated micromyeloblasts (Table 29 f). Penicillin, sulphonamides and repeated transfusions (34 in all) prevented the onset of intercurrent infection during this stage of secondary agranulocytosis (leucocytes between 100 and 300 for a month). Death from heart failure on June 19th 1947.

Pathologically (Professor von Meyenburg) the marrow was reticular and devoid of cells; indeed this condition was so extreme that the pathologist could not have made a diagnosis without the clinical history.

certainly due to urethane but was not stopped by ceasing to give the drug *We should therefore, beware of continuous treatment with urethane, especially as Patterson Webster and others have recorded similar cases*

In our case spleen puncture which had shown considerable retrogression of granulocytopoiesis while arsenic was being given showed even further retrogression after 9 months on urethane while the lymphocytes rose to 48 per cent After a further 9 months (November 1947) there was a striking increase of basophiles (20 per cent) while shortly before death there was increase of immature white cells and great deficiency of neutrophiles and lymphocytes Post mortem puncture showed the presence of many immature cells while histological examination reveals the presence of many young granulocytes in the spleen and marrow without signs of maturation although there was no real myeloid aplasia Presumably therefore urethane had induced complete cessation of cell proliferation in both the spleen and the marrow

The second case treated for 5 months with urethane (Table 29e, No 2) is specially interesting because it is until now the only observation in which the terminal paramyeloblastic change did not start in the spleen but in the marrow as comparative counts clearly demonstrate Thus the splenogram showed few myeloblasts (0.1 per cent) almost all (3.5 per cent) being normal with 68.5 per cent of mature neutrophiles At the same time the sternal marrow contained 57.5 per cent of immature cells 46.3 per cent being paramyeloblasts and promyelocytes But as the hæmogram shows there were no paramyeloblasts in the blood at this stage they suddenly appeared 6 days later reaching 383 000 shortly before death Autopsy (Professor von Meyenburg) confirmed our finding of an almost completely paramyeloblastic marrow and a spleen typical of chronic myelocytic leucosis i.e. filled with strikingly mature cells

This case demonstrates that the terminal dedifferentiation may commence in the spleen or in the marrow As we have emphasised before granulocytopoiesis must be of leukæmic type in both organs not only in the spleen as some writers have suggested Also it is clear that such terminal paramyeloblastic exacerbations are identical with primarily acute leucoses In these cases also there is an initial infiltration of the marrow with paramyeloblasts before many such cells can be found in the circulation it is so to speak an acute aleukæmic paramyeloblastic leucosis The irruption of these cells into the blood is a secondary manifestation Obviously there is not necessarily much involvement of extramedullary foci in these cases although Rohr (2) assumed that this was always present His view is probably true of the chronic myeloid type because the cells do not grow in an aggressive manner whereas in the paramyeloblastic form as in this case and in similar forms without leukæmic

It is conceivable that treatment in an early stage *before* normal haemopoiesis has been destroyed may result in the destruction of all leukæmic cells—that means a cure *e.g.*, with some of the newer derivatives of aminopterin

(f) DISCUSSION AND SUMMARY

It has been clearly shown that there are considerable differences of susceptibility to urethane in different haematological cells and different animal species. On the whole lymphocytes are the most susceptible cells in the normal organism *but the pathological granulocytes of chronic myeloid leukaemia possess a peculiar and specific sensitivity—this is regarded as further evidence of their neoplastic character.* Oddly enough the normal granulocytes of the cat are almost as sensitive as are the pathologically deranged neoplastic cells in human leukaemia

What can be the basis of the great differences of susceptibility?

Todd Plentl Schoenheimer and Kelemen have suggested that urethane interferes in the synthesis of nucleoproteins and so indirectly with mitosis. The differences in susceptibility would then be explained by differences in the composition of cytoplasm and nuclei—and it would have to be assumed that the composition of pathological cells is different from that of normal stem cells. Thus the action of urethane would be analogous to that of stilbamidine (Snapper (2)) and antimony (Rubinstein) on myeloma cells which directly interfere with the synthesis of nucleoproteins. We are not however convinced by these arguments.

Our observations have shown that urethane has a stimulating as well as an inhibitory effect on cellular proliferation. We have already mentioned the preliminary phase of stimulation in the beginning of the treatment—and also the rise of lymphocytes that precedes a fall in normal persons while the same is true of experimental animals including mice (Moeschlin (20)). In tissue cultures Bucher observed increased mitotic activity when urethane was first added. We feel therefore that urethane must be regarded as having a direct or an indirect action on cell division—and this idea is supported by the observation that urethane can induce tumour formation in animals (Henshaw Nettleship Claus Jaffe Moeschlin (20 21)). Further Vogt (1948) has been able to induce both recessive and dominant mutations in *drosophila* with urethane. Then also the changes that urethane induces in the structure of mitoses must not be forgotten.

These three facts—stimulation before inhibition—tumour formation—and structural changes in mitosis—seem to suggest a direct or indirect action of urethane on cell proliferation *i.e.* on the latter end of division. This is much like the view of Dustin Ludford and Moellendorf that urethane is a direct mitotic poison

The spleen was practically an empty reticular framework in which neither lymphocytes nor myeloid cells could be found

The lymphatic tissue of the glands spleen and tonsils showed advanced atrophy Spleen 1.8 kg liver 2.5 kg The only persistent sign of a pre existing leucosis was the presence of hæmorrhages

Before treatment with urethane the blood picture was aleukæmic but the spleen was infiltrated with 97 per cent of paramyeloblasts while as an indication of the presence of congenital hæmolytic anæmia, there were some macro erythroblasts and macrophages containing hæmosiderin The sternal marrow was also almost replaced by paramyeloblasts (88 per cent) After 87 g of urethane had been given the abnormal cells almost disappeared from the blood spleen and liver for about 3 weeks But the exact nature of the cells could not always be ascertained because of their close resemblance to small reticulum cells Histologically all that was left were a few reticulum cells lying in the almost empty meshes of those organs that had been packed with leukæmic elements This case is very similar to one described by Schwarz Tien

TABLE 29f

Myelogram and Splenogram in Acute Myeloid Leucosis before and after Treatment with Urethane

	Sch H aged 26 male			
	Sternum		Spleen	
Erythroblasts	10.6	2.8	—	0.4
Paramyeloblasts	87.6	95.6	97.4	98.4
Myelocytes immature	—	—	—	0.2
Myelocytes mature	0.6	1.4	0.4	0.2
Neutrophiles staff	0.4	0.2	1.4	0.2
Neutrophiles segmented	—	—	0.4	0
Eosinophiles	0.6	—	0.2	0.6
Basophiles	0.2	—	—	—
<i>Hæmogram</i>				
Leucocytes	102 400	3 000	102 400	3 000
Paramyeloblasts	854	70	854	70
Myelocytes immature	3	—	3	—
Myelocytes mature	24	—	24	—
Neutrophiles staff	54	16	54	16
Neutrophiles segmented	3	10	3	10
Eosinophiles	1	1	1	1
Basophiles	—	1	—	1
Monocytes	4	2	4	2
Lymphocytes	—	—	—	—
Thrombocytes	38 000	—	38 000	—

These 2 cases show that urethane can occasionally suppress proliferation of the abnormal cells completely although there is no revival of normal blood formation Then life can only be preserved for a time by chemotherapy and repeated transfusions

NITROGEN MUSTARD

As far as we know there are no reports of the effects of this substance on the cellular composition of spleen films. Block (1948) has reported on the histology of material obtained by spleen puncture in a case of chronic myeloid leukaemia. He found no changes 44-96 hours after an injection of 0.1 mg/kg—there was no clinical improvement. On the other hand he found a distinct decrease of lymphocytes in the glands in a case of lymphatic leucosis.

The clinical effects which closely resemble those of radiotherapy, have now often been described (e.g. Karnofsky). Aleksandrowicz *et al* found that of the hemic elements the granulocytes, lymphocytes and thrombocytes were the most sensitive while erythroblasts were much less so. Reticulum cells increase in number and in phagocytic activity.

TABLE 29g
A. E. aged 66 male

	Before treatment			Four weeks after treatment with nitrogen mustard		
	Hemogram	Splenogram	Myelogram	Hemogram	Splenogram	Myelogram
Erythrocytes	3.38 mill	—	—	2.87 mill	—	—
Leucocytes	78 000	—	—	30 400	—	—
Thrombocytes	51 000	—	—	42 000	—	—
Lymphoid retic. cells	—	—	—	—	—	0.8
Erythroblasts	—	11.8	3.0	1.0	11.0	13.0
Myeloblasts and promyelocytes	1.0	3	4.8	0.5	1.2	3.4
Total immature granulocytes	13.0	56.0	33.6	10.5	3.6	0.8
Neutrophiles, staff	40.0	17.8	34.8	1.0	24.4	34.4
Neutrophiles, segmented	3.5	10.2	21.6	55.5	24.0	27.2
Eosinophiles	5.5	2.0	2	0.5	2.0	1.0
Basophiles	6.0	0.6	3.4	10.5	1.6	2.2
Monocytes	1.0	—	—	0.5	—	0.2
Lymphocytes	1.0	1.6	1.4	1.5	2.4	0.4

We have investigated a man of 66 with chronic myeloid leukaemia who had 11 mg of nitrogen mustard after having been treated with arsenic for 4 years and later with urethane. As he had become refractory to these methods we waited for 2 weeks and then tried nitrogen mustard. Table 29g shows the blood pictures before and 4 weeks after the commencement of the treatment together with the myelograms and splenograms. The general effect was a reduction of immature forms in both the marrow and the spleen being rather

In analogy with inhibition of respiratory ferments (Keilin and Hartree, 1939) and blockading of the sulphonamide effect on bacterias by urethane (Allgower) the effect of this substance may well be supposed to inhibit or destroy some enzyme system essential for cell proliferation. If so, it would follow that the remarkable susceptibility of the stem cells of some groups was due to possession of specially urethane sensitive enzymatic processes.

Investigations on these lines may throw light on the metabolic processes characteristic of neoplastic cells. Quastel showed that urethane has a special action on tissue respiration while Bock found a distinct decrease in the utilisation of oxygen by tissue cultures after addition of urethane. This is specially significant because Schulze has called attention to the fact that normal cellular respiration is pre requisite for normal cell division (Fisher and Stern, Ormsby and Fisher).

Arsenic also decreases the intra cellular respiration of some cells by combining with sulphhydryl radicles (Welfare).

Brock, Ducrey and Herken added 165 gamma/c.c. of urethane to tissue slices and to sea gull eggs—and found that *after preliminary increase of oxygen consumption* there was a distinct fall. This is in conformity with our observations on the initially stimulating effect of urethane on hemopoietic cells.

A difficulty in accepting the view that urethane acts by inhibition of enzyme systems is the lengthy persistence of its effects after its administration is stopped. Thus in a case of Parkinsonism Moeschlin and Meili saw the first increase of granulocytes 18 days after cessation of urethane. Even if the period of maturation of granulocytes (8 days) is subtracted (Moeschlin (12-15)) there are still 10 days during which granulocytopoiesis remains on a reduced level. The same is true of the fatal cases of panmyelophthisis due to urethane.

It seems unlikely that such persistence is due to individual variations in absorption, excretion or detoxication of urethane. Perhaps this long persistence is in relation with the complete destruction or neutralisation of some essential enzyme systems.

The fact that urethane can induce tumour formation indicates the need for care in its use in man. Certainly in this respect urethane resembles other tumour inducing agents such as arsenic and radium which act by altering the mitotic process—and presumably urethane also acts by a similar mechanism—not as a cytostatic agent (Heilmeyer (6)) as for instance stilbamidine on myelomas.

It is of the utmost interest that arsenic, α rays, radium and urethane (and perhaps some oestrogens) can both inhibit and initiate neoplastic processes. Whatever may be the differences between these agents they have in common the power of interfering with the complicated processes of cell division.

The effects are similar to those of urethane viz reduction of the number of immature cells in the spleen the immature myelocytes and their precursors fell to a third of their previous values while there was a slight increase of erythropoiesis. In the marrow the reduction of immature cells was a good deal less striking. Six weeks after the treatment the mitotic index had risen presumably because a new generation of cells had started to proliferate. Probably puncture at the end of treatment would show diminution of mitotic activity but this is likely to be transient. Here as in the case of urethane it is interesting to find that the effect on the spleen is greater than that on the marrow. No valid conclusions can be drawn until a number of other cases have been investigated by spleen and marrow punctures.

3 PARAMYELOBLASTIC LEUCOSIS

We have already said that we have replaced the concept of acute leukaemia by that of paramyeloblastic leucosis because the disease may run a chronic course or even if it is apparently acute may have developed insidiously over quite a long time.

By *paramyeloblasts* we mean the abnormal sometimes granular cells which gradually replace the granular leucocytes in the blood and marrow and which unlike the cells found in chronic myelocytic leucoses show only a very slight tendency to maturation never attaining the polymorphonuclear stage (Fig 47 c).

A chronic myelocytic leucosis may end as a paramyeloblastic leukaemia in which one proliferating cell type which has lost all power of further differentiation is overgrowing the other cells leading finally to death (Fig 47 d).

The *spleen* is not usually enlarged in the aleukemic stage (Moeschlin and Rohr (3)) but is frequently palpable in sub leukaemic and leukaemic cases. It is interesting to note that in such cases injection of 1 mg of adrenalin intramuscularly is followed by decrease in the size of the spleen and a great rise of paramyeloblasts in the blood e.g. from 70 000 to 700 000 in Hortling's case. As regards the great tendency to bleeding spleen puncture has only been performed in those cases in which there was no undue inclination to haemorrhage (Table 29i).

Our cases were of sub leukaemic type accompanied by definite splenomegaly. In Cases 1, 2 and 4 the infiltration of the spleen with paramyeloblasts was in a very early stage whereas in the third and last case almost all other normal spleen cells had been thrown back by the paramyeloblasts. Mitoses were found in the paramyeloblasts in every case clearly showing that these cells had really been obtained from the spleen.

Paramyeloblasts in both spleen and marrow films were distinctly less mature than those in the peripheral blood i.e. their nuclei and

more marked in the latter. In the sternal marrow mature neutrophils rose by one fifth whereas they were doubled in the spleen. Erythropoiesis increased in the marrow after nitrogen mustard but less than after arsenic or urethane but this did not occur in the spleen.

It was not possible to determine the effect on the mitotic index because the inhibition due to arsenic had not yet worn off (mitotic index 0.9 per 1 000). Three weeks after the last injection of nitrogen mustard the index was 2.2 per 1 000. Probably in cases that have not had previous treatment there would be a fall in the mitotic index.

RADIO ACTIVE PHOSPHORUS

Since Lawrence introduced this isotope of phosphorus into the treatment of chronic leucoses there have been many publications on the subject. Apparently the immediate results are excellent but relapse soon occurs. Table 29h shows the effects of 24 millicuries on the condition of the spleen and marrow in a case that had been treated with urethane 18 months earlier, and with arsenic more recently.

TABLE 29h
P E aged 31 male

	Before treatment			Six weeks after P 37 treatment		
	Hæmogram	Splenogram	Myelogram	Hæmogram	Splenogram	Myelogram
Erythrocytes	2.57 mill			1.93 mill		
Leucocytes	35 400			17 000		
Thrombocytes	47.900					
Macrophages	—	—	—	—	—	—
Plasmacyt retic cells	—	—	0.4	—	—	0.2
Lymphoid retic cells	—	—	2.4	—	—	9.2
Pulp cells	—	—	—	—	1.3	—
Erythroblasts	4	2	1.8	—	9.4	8.0
Myeloblasts and promyelocytes	17	34½	70.0	11.5	12.7	16.7
Myelocytes immature	6½	10	6.4	—	8.0	2.4
Myelocytes mature	3	15½	7.4	—	25.3	2.2
Neutrophiles staff	8	9	14.8	7.0	7.7	9.0
Neutrophiles segmented	13½	10	12.4	10.0	4.3	7.2
Eosinophiles	12½	11	14.7	11.0	8.4	15.8
Basophiles	34½	2½	71.6	43.0	10.7	26.8
Monocytes	—	—	—	—	0.7	—
Lymphocytes	5	4½	3.2	10.5	11.3	3.0
Plasma cells	—	—	—	—	0.3	—

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TABLE 29h
P E aged 31 male

	Before treatment			Six weeks after P 37 treatment		
	Hemogram	Splnogram	Myelogram	Hemogram	Splnogram	Myelogram
Erythrocytes	25.7 mill			19.3 mill		
Leucocytes	35,400			17,000		
Thrombocytes	47-900					
Macrophages	—	—	—	—	—	—
Plasmacyt retic. cells	—	—	0.4	—	—	0.2
Lymphoid retic. cells	—	—	2.4	—	—	9.2
Pulp cells	—	—	—	—	1.3	—
Erythroblasts	4	—	1.8	—	9.4	8.0
Myeloblasts and promyelocytes	17	34½	70.0	11.5	12.7	16.2
Myelocytes immature	6½	10	6.4	—	8.3	2.4
Myelocytes mature	3	15½	7.4	—	5.3	—
Neutrophiles staff	8	9	14.8	7.0	7.7	9.0
Neutrophiles segmented	13½	10	12.4	10.0	4.3	7.7
Eosinophiles	12½	11	14.2	11.0	8.4	15.8
Basophiles	14½	2½	21.6	43.0	10.7	6.8
Monocytes	—	—	—	—	0.7	—
Lymphocytes	5	4½	3.2	10.5	11.3	3.0
Plasma cells	—	—	—	—	0.3	—

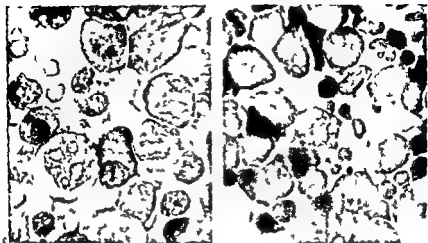


FIG. 47 Spleen films in paramyeloblastic leucoses

(c) Large partially lobulated paramyeloblasts in a case of so-called monocytic leukaemia

(d) Numerous vacuolated types together with erythroblasts and myelocytes indicating that in this case the acute leucosis has to be regarded as being the terminal paramyeloblastic stage of chronic myelocytic leucosis

The differences between the abnormal (neoplastic) cells and those of the normal myeloid series are particularly well seen in micromyeloblastic leucoses. Thus in Case 5 we saw almost naked microparamyeloblastic nuclei (with a few Auer bodies) as well as rather large but normal myelocytes—and no transitional forms. *We consider thus co-existence of a distinct pathological cell stream and the normal though scanty myelopoietic row as further evidence of the neoplastic nature of the leukaemias.*

Mitoses Many beautiful mitotic figures can often be found in paramyeloblasts but as these are abnormal (probably neoplastic) elements the mitoses differ considerably from those of normal myeloblasts and myelocytes.

Isaacs (1930) was the first to describe the occurrence of haploid mitoses in leukaemic cells *i.e.* karyokinesis in which there are 12 instead of 24 chromosomes in prophase or 24 instead of 48 in anaphase (diaster). Groat (1933) as well as Moeschlin and Rohrer (1939) have confirmed this. As such haploid mitoses have only been found in malignant tumours both carcinoma and sarcoma *we consider that their occurrence in the leukaemias is one of the strongest evidences of the neoplastic nature of these diseases.* Certainly we are convinced that such abnormalities of cell division are not corresponding to a hyperplasia or to Naegeli's irreversible disturbance of correlation.

TABLE 29
Paramyeloblastic Leukaemias

Case number	1	2	3	4	5
Age sex	74 ♂	51 r	23 ♂	57 ♂	26 ♂
Cell type	premye- locytes	mono- cytoid	immature myelo- blastic	mono- cytoid	micromye- loblastic
Spleen (in cm)	12	13	20		38
<i>Hæmogram</i>					
Hæmoglobin (in %)	69	31	69	66	60
R C (in millions)	—	—	2 872	3 0	2 66
Platelets	reduced	reduced	4 / — 11 000	27 / — 81 000	reduced
Leucocytes	2 200	6 300	12 900	3 900	23 000
Paramyeloblasts (in %)	17½	87½	61	54½	94½
Paramyeloblasts absolute	385	5 512	7 869	2 126	21 735
Total myelocytes (in %)	1	—	3½	6	1
Neutrophils staff	20	—	8	3	7½
Neutrophils segmented	6	—	12½	9½	—
Eosinophiles	3	—	1	1½	1
Basophiles	4	—	—	1	1½
Monocytes	4	—	1	—	—
Lymphocytes	44	10½	13½	30½	—
<i>Sternal marrow</i>					
Paramyeloblasts ()	40	—	99	—	95 6
<i>Splenogram</i>					
Macrophages	—	0 3	—	0 4	—
Plasmacyt retic cells	1 5	2 6	0 1	2 4	—
Pulp cells	0 1	—	—	1 0	—
Erythroblasts	0 1	0 2	1 2	4 8	0 4
Paramyeloblasts	10 7	78 6	96 2	28 6	98 2
Paramyeloblasts in mitoses	+	0 2	0 2	0 2	0 2
Total Myelocytes	0 4	—	0 9	1 6	0 4
Neutrophils staff	9 1	0 3	0 3	3 2	0 2
Neutrophils segmented	8 2	0 1	0 1	2 0	0 2
Eosinophiles	1 2	—	0 1	2 8	0 6
Basophiles	1 5	—	—	0 6	—
Monocytes	0 8	0 1	0 2	—	—
Total lymphocytes	65 2	62 3	0 5	5 6	—
Plasma cells	1 2	5 6	0 2	—	—

nucleoli were larger while the cytoplasm was narrower (Fig 47 c d)

Histological examination of embedded material from spleen puncture in Case 2 shows clearly that *in the early stages of infiltration the abnormal cells are in a focal arrangement reminiscent of sarcomatous metastasis*. Fig 48 shows paler areas (x) which correspond to collections of monocytoïd paramyeloblasts while the remainder of the spleen preserves its normal structure

In all 5 cases a few erythroblasts and myelocytes were found but the latter did not show any signs of leukæmic abnormality and there were no transitional forms between them and the paramyeloblasts. For this reason we regard their presence as being due to a compensatory myeloid metaplasia following the decrease in size of the medullary spaces by proliferation of the abnormal cells (p 61)

there are any pathognomonic changes in the blood or spleen. If however the marrow is not characteristic of myeloid leukaemia although some cells resembling paramyeloblasts are found in the blood the possibility of a reticulosis or lymphosarcoma must be considered (p. 176). Then spleen or gland puncture may confirm the diagnosis.

(For an account of the possible effects of urethane in para myeloblastic leukaemia see the previous chapter.)

4 POLYCYTHÆMIA VERA

We have included this disease among the hemoblastoses because modern opinion basing itself on the analogy of the leukaemias tends to regard it as being a non malignant neoplastic proliferation of the erythroblastic system (Weber Minot and Buckman Noll and Benaroiu Rohr) in which the parent cells like those of chronic myelocytic leucosis have retained a good deal of their power of maturation. Admittedly Heilmeyer and Apitz do not agree with this view.

The spleen is usually enlarged and Heilmeyer attributes this to several factors viz. increased reservoir function, increased hæmolytic activity and least of all to the presence of ectopic foci of hemopoiesis.

We have no personal experience of spleen puncture in this condition. Weil (1936) did not find any striking increase of erythroblasts, an observation that supports Heilmeyer's view. Weil cites the following as a typical splenogram —

Erythroblasts 5 per cent, premyelocytes 2 per cent, neutrophils 46 per cent, monocytes 3 per cent, and lymphocytes 44 per cent.

Dedichen (1941) recorded the case of a man of 44 with hæmoglobin 109 per cent, red corpuscles 6.11 millions and 24 000 leucocytes of which 5 per cent were myelocytes. In spleen films there were many myelocytes, a few megakaryocytes and many erythroblasts. The photographs that accompany his report as well as the myelogram which revealed a hyperplastic but not specially erythroblastic marrow make it clear that this was in fact a case of chronic myelocytic leukaemia. It is recognised that polycythæmia may occur in the early phases of this malady (Naegeli (1) Schwarz 1939 and Case 13 on p. 174).

In another case of a woman of 44 with typical polycythæmia Dedichen's spleen puncture revealed only lymphocytes and occasional erythroblasts but neither myelocytes nor megakaryocytes.

There is no doubt that transitions between polycythæmia vera and chronic myelocytic leucosis do occur (Naegeli Rachmilewitz etc.). On p. 174 we have described such a case in which erythro leukaemia developed.



FIG. 43 Focal arrangement of paramyeloblasts (P) in the spleen (analogous to metastasis of a neoplasm) in the early stage of a case of acute paramyeloblastic leucosis (section from embedded spleen puncture material)

In addition to haploid mitoses other abnormalities of division we have observed in the spleen marrow and not rarely in the peripheral blood (Andres and Shiwago) multipolar mitoses and clumping fragmentation or a splintering off or thickening of the chromosomes

Diagnostic Significance Spleen puncture plays no part in the diagnosis of paramyeloblastic leucoses. Sternal puncture is far more useful and may indeed allow of a diagnosis being made before

myeloid cells are numerous with predominance of erythropoietic elements associated with high erythroblast values in the marrow. *As the erythroblasts in spleen films from chronic myeloid leucoses may be as high as 33 per cent only cases in which there is also great proliferation of erythroblasts in the marrow should be classified as erythroleukæmias*

We have 3 cases in which spleen puncture was performed (see Table 22 Case 11-13)

Case 11 A F female aged 42 housewife

Increasing weakness for some years In April 1943 enlargement of the spleen and liver was noted hæmoglobin 52 per cent leucocytes 64 200 with many myelocytes and erythroblasts Admitted to hospital June 9th 1943

On examination Enlarged glands Spleen firm and palpable 17 cm liver somewhat enlarged 12 cm. in the parasternal line B S R 12 mm protein 6.0 prothrombin 60 per cent Takata negative Weltmann 0.2 serum iron 65 gamma

Blood Picture Red corpuscles 3.5 million hæmoglobin 70 per cent leucocytes 43 300 reticulocytes 2.3 per cent normoblasts 290 myeloblasts 7½ per cent premyelocytes 2½ per cent myelocytes 9 per cent immature myelocyte 7 per cent neutrophils 20½ per cent segmented 27½ per cent basophile 12½ per cent monocytes 43 per cent and lymphocytes 8 per cent platelets 220 000

Sternal Puncture (June 23rd 1943) Erythroblasts 4.2 per cent myeloblasts 1 per cent myelocytes 29.3 per cent neutrophils 47.3 per cent

Arsenic was administered and the leucocytes decreased

November 1943 Gradual increase of erythroblasts in the blood (November 24th 1943) Red corpuscles 2.5 million hæmoglobin 65 per cent leucocytes 19 100 erythroblasts 3.250 myeloblasts 4½ per cent myelocytes 2½ per cent

Spleen Puncture (December 28th 1943) See Table 22 Great increase of erythroblasts (59 per cent) while there were 38.3 per cent in the sternal marrow with 16.9 immature granulocytes March 23rd 1944 second spleen puncture (see Table 22)

The leucocytes were kept between 10 000 and 13 000 by arsenic until death occurred on April 19th 1944

Autopsy confirmed the diagnosis of chronic myelosis with granulocytic and erythroblastic infiltrations of liver spleen glands and a distinct increase of the erythroblasts even in the bone marrow

This was thus a typical case of chronic erythroleukæmia in which there were 3 250 normoblasts in the blood and up to 59 per cent of erythroblasts in spleen puncture so that the 4 per cent of myeloblasts and 11 per cent of myelocytes were almost overshadowed (Fig 49)

Proliferation of erythroblasts was also well seen in the marrow where red cell formation is diminished in ordinary cases of leukaemia There was distinct increase of basophilic erythroblasts unlike the condition in myelocytic leucoses

In the second case (Number 12) we were dealing with a woman of

5 ERYTHROLEUCOSIS AND ERYTHROBLASTOSIS

We owe the concept of erythroleukemia to di Guglielmo who was the first to publish reports of the disease. Since then a number of cases of concomitant proliferation of the erythropoietic and leucopoietic tissues has been recorded. Sometimes the former becomes more and more pronounced so that a case of chronic erythroleukemia may end as one of acute erythroblastosis (*vide* Heilmeyer and Schöner)

Pure erythroblastosis (*erythræmia acuta* of di Guglielmo) are probably much rarer than are erythroleukemias which form 2 per cent of the leucoses seen in our clinic. In the literature up to 1939 (Moeschlin (1)) we found 5 proven cases, and further search has revealed records of 9 more (Israelis 1939, Barcaglia, 1939, Quattrin 1939, Roth 1940, Nabholz 1942, Lanza 1940, Duvois 1941, Kienle 1943, Poli 1947, Denolin 1949). As far as we are aware there are no records of spleen puncture in pure erythroblastosis.

The cases published by Weil (3) in 1938 under the name 'crypto erythroblastosis' were certainly not examples of genuine erythroblastosis. The great splenomegaly and the increase of megakaryocytes in spleen films make clear that some of Weil's cases were either aleukæmic or subleukæmic examples of chronic myelocytic leukemia. In other cases he appears to have been dealing with a special form of reaction to infection especially tuberculosis of the spleen with many erythroblasts and myeloblasts in spleen films.

In one of his female patients there had been great splenomegaly for 16 years with 6.5 million red corpuscles, 35 000 leucocytes and occasional erythroblasts and myeloblasts while spleen films contained large numbers of immature granulocytes and erythroblasts. Autopsy revealed typical tuberculosis of the spleen in which Koch's bacilli were demonstrated.

It is not clear whether this was an example of primary tuberculosis of the spleen (with consequent formation of hemopoietic foci) as in our own cases or whether tuberculosis was an intercurrent infection in the course of chronic myeloid leukemia. The latter is a fairly common terminal event which we have been able to observe three times.

The fact that the leukæmic blood picture persisted after splenectomy in Weil's cases is strong evidence against their being of inflammatory nature. We much prefer the term 'myeloid reaction associated with infective splenomegaly' to 'crypto erythroblastosis' which we consider totally misleading (see p. 108). Nordenson (1946) has recorded 3 cases which he reckons as examples of the disease described by Weil. In 2 cases spleen puncture films contained many erythroblasts and myelocytes but as neither splenograms nor myelograms are given exact classification is not possible.

Spleen punctures in erythroleukemia (excluding Weil's (3) dubious cases) have been recorded by Bianchi (1939), Forconi (1939) and Heilmeyer and Schöner (1941). They all agree that

ment had to be given because of the discomfort due to the enormous size of the spleen although the characters of the blood picture itself would not have necessitated irradiation. This patient has been under our observation now for five years and if one were not acquainted with the earlier findings one would now regard the condition as being pure chronic erythroblastosis.

This case is of particular interest inasmuch as it demonstrates the close relationship between polycythæmia, chronic myelocytic leucoses and erythroleukæmia so that all these three diseases can be regarded as being variants of one and the same pathological process. In our cases of erythroleukæmia we found many degenerative changes in the erythroblast mitoses e.g., complete pyknosis of the chromosomes and cells with detached fragments of chromosome material etc. These changes are presumably due to the leukæmic nature of the affected cells but it has to be remembered that similar alterations have been described by Fieschi and also Kienle in cases of toxic damage to the marrow.

In every case there was a distinct excess of mitoses in the polychromatic erythroblasts while the mitotic curve showed an excess of monaster types. This does not differ appreciably from the proportion of erythroblast mitoses of the mitotic curve in normal marrow (Fieschi, Kienle).

It may be said therefore that spleen puncture in our 3 cases of erythroleukæmia showed well marked proliferation of erythroblasts particularly of the immature forms the numbers of which exceeded those of the white cells although these also showed overgrowth.

Spleen puncture in these cases is likely to be of diagnostic value only in those cases in which no marrow can be obtained by sternal puncture and in which the presence of osteosclerosis might arouse a suspicion of a myeloid reaction due to the formation of extra medullary myeloid foci. The number of erythroblasts in spleen puncture in cases of non leukæmic origin never exceeded 20 per cent while the number of myelocytes in inflammatory conditions was never higher than 3 per cent. In osteosclerotic anæmias (p. 66) the myelocytes might be as high as 25 per cent but the lymphocytic structure of the organ (up to 60 per cent) was preserved as compared with the striking diminution of lymphocytes in erythroleucoses. *It is probable therefore that purely reactive myeloid hyperplasias can be distinguished from leukæmic diseases by this quantitative relationship.*

6 EOSINOPHILE BASOPHILE AND MEGAKARYOCYTE LEUCOSES

As in the case of erythroleucoses it is obvious that there are no pure leukæmic proliferations of a single cell type. Thus there are innumerable variants between typical myelocytic leucoses and cases

61 who showed well marked enlargement of the liver and spleen. In the blood the leucocytes reached 20,000 with 1,570 erythroblasts and 2 per cent of immature myelocytes. In spleen puncture there were 41 per cent of erythroblasts but only 13.6 per cent of immature granulocytes. Unfortunately we were never able to aspirate any bone marrow. This failure was apparently due to patchy osteosclerosis which was well shown by X rays. At autopsy hyperplastic red marrow was found in the long bones and the histological appearances were typical of erythroleukemia. In a third case (Number 13), a man of 54 presented an enormous swelling of the spleen (42 cm) which extended into the right iliac fossa. The red corpuscles were 6.2 million with 118 per cent hemoglobin, the leucocytes at this time were 15,000 with 4.5 per cent myelocytes and 3,800 erythroblasts.

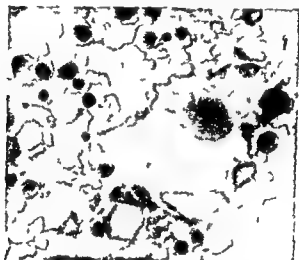


FIG. 49 Erythroleukemia with large numbers of erythroblasts and one megakaryocyte (spleen puncture)

Spleen puncture showed general infiltration of the spleen with erythroblasts of which there were 53.5 per cent and only 20 per cent of myeloid cells (Table 22). The sternal marrow showed a mainly erythroblastic hyperplasia with 283 erythroblasts to every 100 white cells (73 per cent erythroblasts). As the disease developed the erythroblasts became more numerous and less mature. Treatment with arsenic appeared to inhibit the abnormal erythroblasts and in spleen puncture they fell from 54 per cent to 23 per cent in a month while the spleen diminished in size from 42 cm to 27 cm. Within 5 months the spleen became enlarged again while the erythroblasts in the splenogram rose to 71 per cent and no myelocytes could be found. Careful irradiation which had to be repeated every 6 or 8 months succeeded in reducing the size of the spleen and in diminishing the percentage of erythroblasts in it to about half. The treat-

In some cases of reticulosis abnormal globulins similar to those found in association with myeloma are produced by the neoplastic cells (p 127) A hyperproteinemia may result and the Takata cephalin and cadmium reactions can be positive while the B S R is greatly increased and in some cases there is a pathological electrophoretic pattern (Wuhrmann and Wunderly)

Waldenstrom (1948) has recently described a new disease

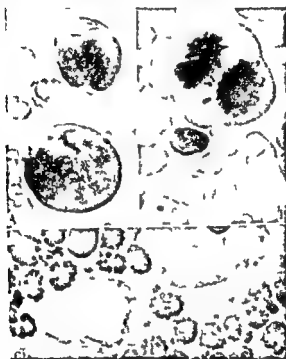


FIG 50 a b c *Reticulosis of spleen and liver* (Female aged 46)

(a) Rather large lobulated cells with small indistinct nucleoli a compact chromatin is characteristic of the cells of the reticuloses (Spleen puncture)

(b) Mitosis in such a cell rather short thick chromosomes

(c) *Phase contrast picture* The absence of distinct nucleoli is characteristic This should be compared with the cells of lymphosarcoma and other sarcomas (Zeiss phase contrast microscope 1 000)

having a distinct difference to myeloma associated with macro globulinemia and infiltration of the marrow with small cells resembling lymphoid reticulum cells while a protein of high molecular weight was present in the blood

These observations indicate how important is the part played by the reticulo endothelial system in the form of certain protein substances It seems to us probable that those reticuloses in which there is distinct formation of reticular fibrils are usually not associated with changes in the serum proteins The power of synthesis

in which there is great predominance of one particular cell type

We have seen cases in which it has only been during the course of the disease that cells of eosinophile or basophile series have greatly overwhelmed those of other types

From the point of view of differential diagnosis the only cases that present difficulty are those of the syndroma of chronic eosinophilia with splenic enlargement Heilmeyer has shown that leukemic eosinophilia can be distinguished from the non leukemic type by the action of urethane in the former Also we have no experience of spleen puncture in eosinophile leukemias and have been unable to find any in the literature We should assume that spleen puncture would be useful in diagnosis and we should expect to find many immature eosinophiles myelocytes and distinct reduction of lymphocytes (see also p 133) Perhaps in the non leukemic type of splenomegalia with chronic eosinophilia the number of lymphocytes would still be elevated in comparison with the leukemic form corresponding to our findings in osteosclerosis and myelofibrosis

We also have no personal knowledge of spleen puncture in true megakaryocyte leucoses and none appear to have been recorded We should however expect to find many of the more immature precursors of these cells The same is presumably true of the rare condition of thrombocytosis (thrombocythæmia of Mortensen and Hemmeler) which may also be associated with splenomegaly

7 PRIMARY MALIGNANT RETICULOSES AND RETICULO- ENDOTHELIOSES

It is necessary to discuss the concept of primary malignant reticuloses because of the confusion of names used in the literature We include these conditions in our section on hemoblastosis because by the term primary malignant reticuloses we mean a neoplastic condition in which there is a proliferation of undifferentiated cells of the reticulo endothelial system which does not remain localised and in which such cells may appear in the blood it is therefore possible to distinguish leukæmic and aleukæmic reticuloses (Uehlinger)

Most of the publications that have appeared on this subject have been on a pathological and anatomical basis without examination of the marrow glands or spleen by the method of puncture It is however impossible by the anatomical method to separate a paramyeloblastic leucosis from a small celled sarcoma or a reticulosis except in those cases where there is distinct formation of reticular fibres Even so genuine reticuloses do occur in which the power of forming fibrils has been lost The presence or absence of such fibrils depends on the type of proliferating reticulo endothelial cell and its degree of differentiation

system can occur in infective conditions (Uehlinger Deelmann) It is therefore reasonable to speak of primary neoplastic malignant reticulosis and on the other hand, also of infective reactive secondary reticulosis (Uehlinger Glanzmann) In one case of Still's disease (p 98) we observed such a secondary reticulosis in which there was great increase of lymphoid reticulum cells in the sternal marrow (99 to every 100 granulocytes) while plasmacytoid reticulum cells were greatly increased (14.4 per 100 granulocytes) In the spleen only the plasmacytoid reticulum cells were increased the other reticulum cells showing no changes The problem of infective reactive reticulosis has been discussed in the section on infectious mononucleosis (p 87)

Deelmann would place many of the cases that we have called primary reticulosis into a separate group which he classes as being reactive in nature and places between the true inflammations and the true neoplasias It is of course always possible as in the case of leucoses that certain so called neoplastic forms are really due to infection by a virus

The numerous cases of so called monocytic leukaemia that have been published show how much care is needed in separating this condition from the primary reticuloses and also that it is not sufficient to depend upon the blood picture and the histological appearances Thus by sternal puncture in all our cases we were able to detect the origin of these cells from typical promyelocytoid paramyeloblasts (Moeschlin and Rohr(3)) and not from reticular endothelial cells We do not however doubt the existence of a true monocytic reticulosis originating from the reticulo endothelial system We would however emphasise as we have done with Rohr that the diagnosis of a genuine reticulo endothelial monocytic leukaemia is never convincing in the absence of sternal puncture

It has already been mentioned in the discussion of lymphatic leukaemia that some of the cases are really reticuloses especially those with a remarkable cell polymorphism and atypical cell forms in those forms in which there is a close resemblance to the structure of lymphocytes when definite diagnosis is impossible These conclusions are not practically important because the general view is that these diseases are neoplastic in nature and dedifferentiation would then affect the reticulum cells and the elements transitional between them and the lymphatic myeloid and erythroblastic elements (Apitz Rohr Heilmeyer) But in all such cases an increased B S R and other changes in the blood proteins suggest a reticulosis

Storage diseases of the reticulo endothelium will be discussed in a separate section

We have records of 7 cases of primary reticulosis in which spleen puncture was performed In all of these it was possible to exclude lymphatic and myeloid leucoses because of the structure of the

ing proteins by such cells manifests itself in the formation of a more or less fixed protein product, whereas in the reticuloses in which fibrils are not formed changes are to be expected more frequently in the blood proteins because these cells may under certain circumstances, deliver their proteins into the blood stream

In our opinion a diagnosis of true reticulosis is most likely to be made by examination of cells in films of marrow glands spleen and perhaps liver, while in a few cases the characteristic cells may be found in the blood. There is no doubt that this method is much less misleading than that of fixation with the consequent shrinkage of cells

Morphology : The cells of primary reticulosis as seen in films have a fine granular but remarkably close and compact chromatin as compared to those of lymphosarcomas or paramyeloblastic leucoses (Figs 40 50 a, 51 a c, 52 e f and 53). The nuclear structure bears some resemblance to that of myeloma cells and also that of glandular fever cells. The nucleus is more or less round and often shows a tendency to indentation or even lobulation. As compared with paramyeloblasts and lymphosarcoma cells the nucleoli are smaller and scantier (usually 2-4) and may even be absent. The cytoplasm is often very narrow (Figs 40 and 51) but occasionally is relatively bulky (Fig 53) and according to the stage of maturation shifting from intense basophilia to a greyish colour and as in the case of other neoplastic cells there may be numerous vacuoles. In Case 5 the cells had relatively dark blue cytoplasm and closely resembled myeloma cells whereas in Case 3 (Fig 50) their appearances were those of genuine large atypical reticular monocytes. In Case 6 there was some resemblance to atypical lymphocytes.

Mitoses are usually relatively numerous. The chromosomes being rather thick and nearly touching one another at an acute angle (Fig 51 e).

In some cases even examination of puncture fluid does not permit of a definite classification of the cells and these cases have to be recorded simply as hemoblastoses or leukoblastoses. Sometimes the striking size and density of the nuclei or the extreme basophilia of the cytoplasm enables a diagnosis of reticulosis to be made especially when the sternal marrow shows no proliferation of these cells. If however there are no distinctive structural changes and if all the blood forming tissue is affected it may be impossible to distinguish the condition from an ordinary paramyeloblastic leucosis or sarcomatosis. Changes in the blood proteins then may point to the diagnosis of reticulosis.

The finding of reticulo endothelial cells in the blood even if accompanied by proliferation of such elements in the tissues is not sufficient grounds on which to base a diagnosis of primary reticulosis because reactive proliferations of the reticulo endothelial

of firm consistence. The tonsils were not enlarged, the spleen was enlarged to percussion but was not definitely palpable. The liver was not enlarged. W.R. negative. B.S.R. 41 mm. serum protein 5.4. Takata reaction negative. Weltmann reaction normal. serum iron 80. bilirubin 1.9. X rays of the pelvis and stomach showed no abnormality but there was a mass in the region of the hilum of the right lung. Fractional test meal showed histamine refractory achylia.

Blood Picture Hemoglobin 51 per cent, red corpuscles 2.4 million, reticulocytes 2.2 per cent, leucocytes 4,300, myelocytes 1 per cent, staffs 17.5 per cent, polymorphs 33 per cent, eosinophiles 0, basophiles 1.5 per cent, monocytes 21 per cent, lymphocytes 12.5 per cent, tumour cells 15 per cent, platelets 32,500. Red corpuscles showed anisocytosis and polychromasia.

Structure of the Tumour Cells These showed considerable variation in size, some being as small as old lymphocytes while others were as large as neutrophils. The cytoplasm was quite narrow and was pale blue and many cells appeared to consist of naked nuclei. The chromatin was strikingly close and homogeneous quite unlike that of myeloblasts or young lymphocytes. Some of the nuclei showed distinct indentations and lobulations, the latter always lying close together and at first glance appearing as if they were splits in the nucleus (Fig. 40). Unlike the cells of lymphosarcoma or paramyeloblasts very few of these elements possessed one or two indistinct nucleoli. The smallest of these cells could not always be distinguished from old lymphocytes but as a rule the striking density of their nuclei enabled the distinction to be made. Even in the peripheral blood occasional mitoses were seen (Fig. 51 e).

TABLE 30

Hæmogram					Splenogram									
Leucocytes (in 1000)	Neutrophils staff	Neutrophils segmented	Lymphocytes	Tumour cells	Macrophages	Plasma vt. retic. cells	Pulp cells	Erythroblasts	Myelocytes	Neutrophils staff	Neutrophils segmented	Basophiles	Monocytes	Lymphocytes small
16	25	39	12	1	0.3	0.1	4	0.7	0.7	71	70	0.7	7.5	56.4
														4.8
														62
														0.6
														91

Spleen Puncture (Table 30) In addition to the cells of normal spleen puncture there were a few erythroblasts (0.2 per cent) and myelocyte (0.4 per cent) together with 91 per cent of the abnormal cells already described. On the whole these cells were rather larger than those in the blood and their lobulation was more striking. In sections from spleen puncture material it is possible to recognise the presence of large numbers of cells with indented and lobulated nuclei composed of rather dark chromatin. These almost fill the pulp and only a few lymphocytes, myelocytes and erythroblasts could be seen lying between them (Fig. 51 b). The reticular fibrils are greatly increased.

Sternal Puncture Macrophages 0.2 per cent, lymphoid reticulum

characteristic cells and sometimes by the distribution of reticulum fibrils or by changes in the serum proteins. A typical case is recorded in detail.

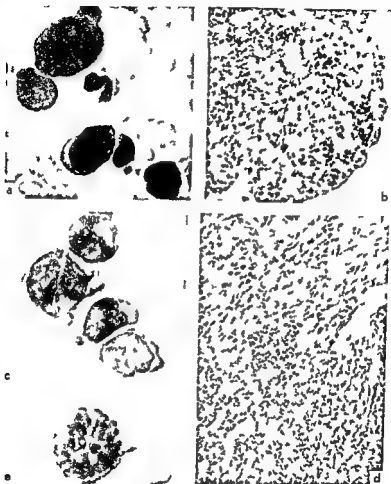


FIG. 51. Primary acute reticulosis (reticulo-endotheliosis).

(a) Typical pathological reticulum cells in spleen films (see also coloured Plate II Fig. 40).

(b) Section from spleen puncture material showing increased cellularity and increase of reticulum fibrils.

(c) Pathological reticulum cells. (Gland puncture films from a case of primary acute reticulosis.)

(d) Section from an excised lymph gland. The normal structure has completely disappeared; tumour cells infiltrated the capsule and there is considerable increase of reticulum fibrils.

(e) Blood film. Mitosis of a typical pathological cell of the same type as in the spleen and glands.

Case 1 B K. Housewife aged 74 admitted to hospital August 25th 1943 with a history of rheumatism at the age of 43 and of recently increasing fatigue and occasional fainting attacks.

On examination there was distinct pallor and aortic incompetence; practically all the lymphatic glands were about the size of hazel nuts and

system including the spleen was mainly affected while the liver was only slightly involved and the marrow remained normal (Fig 52 *a b*) The proliferating abnormal cells were found in considerable numbers in the blood (650 cells per cmm i.e. 15 per cent of the total nucleated cells) The condition has however to be regarded as a sub-leukemic form of reticulosis in which there was histological formation of reticular fibrils in the spleen and glands (Fig 51 *b* and *d*)

Clinically the case was characterised by gradually increasing enlargement of the spleen and particularly of the lymphatic glands accompanied by anaemia and reduction of serum protein The B S R was distinctly increased and X rays did not reveal changes in the bones

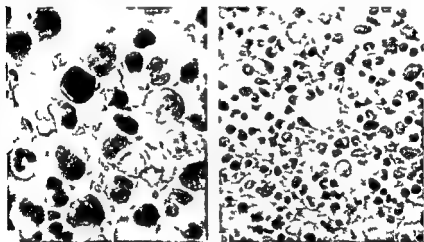


FIG 52 *a b* : Stereal marrow of primary acute reticulosis
Note the normal marrow in contrast to paramyeloblastic leukæmia
(*a*) smear (*b*) section no increase of the reticulum

The condition of the marrow was such as to exclude a paramyeloblastic leucosis with certainty while lymphatic leukæmia could also be excluded because of the structure of the cells and the formation of reticular fibrils Furthermore the histological appearances in the spleen and liver were not those of lymphatic leukæmia Even the young cells had a strikingly close and homogeneous chromatin with distinct indentations and lobulations of the nucleus unlike those found in any case of lymphatic leukæmia

The fact that the pathological anatomist at the autopsy recorded the condition as being paramyeloblastic leucosis is explained by the fact that the details of the nuclear structure could not be recognised in post mortal and fixed tissues Especially the post mortal autolytic changes in the bone marrow made a correct examination difficult

cells 0.8 per cent tumour cells 4 per 100 white cells Erythro
 blasts basophile 6.4 per cent polychromatic 35.8 per cent myelo-
 cytes 0.4 per cent myelocytes immature 8.2 per cent half mature
 18 per cent mature 2.2 per cent metamyelocytes 6 per cent neutro-
 phils staff 43 per cent segmented 7.4 per cent eosinophiles 2 per
 cent basophiles 0.6 per cent monocytes 3 per cent lymphocytes
 9.2 per cent (Fig 52 a) This shows slight shift to the left with a few
 pathological cells of the same type as those found in the blood glands
 and spleen

In sections from the marrow puncture material it was found that the
 structure was practically normal and that there were no focal infiltrations
 (Fig 52 b) or increase of reticulum cells

Puncture of a cervical gland was difficult because of the dense con-
 sistence. In films no lymphocytes were found practically all the cells
 being of the pathological type already described but with even more
 striking variations in size and lobulation

Sections of an excised cervical gland showed that the cells formed a
 rather reticular but closely woven tissue. The fibrils stained faintly red
 with Van Gieson and were well stained with silver. In the peripheral
 parts the protoplasm of the cells were rather scanty while the nuclei
 were here rather larger than those of the cells in the centre. There were
 many mitoses and occasional giant cells. The tumour had broken through
 the fibrous capsule in several places

A histological diagnosis (Professor von Meyenburg) of *reticulum
 sarcoma* was made (Fig 51 d). Treatment consisted of repeated blood
 transfusions which produced slight improvement. The leucocytes varied
 between 2 500 and 4 800 with 8-15 per cent of pathological cells. The
 patient was discharged on October 23rd 1943 with the hæmoglobin up to
 63 per cent. This had fallen to 51 per cent by the middle of December
 when the glands had also increased in size. She was re-admitted on
 January 16th 1944. Her general condition was bad hæmoglobin
 33 per cent red corpuscles 1.2 million leucocytes 7 400 1 normo-
 blast per 200 white cells myelocytes 2 per cent staffs 15.5 per cent
 polymorphs 20.5 per cent eosinophiles 0 basophiles 0.5 per cent
 monocytes 12 per cent lymphocyte 18 per cent tumour cells
 32 per cent. The spleen was palpable and firm and all the superficial
 lymphatic glands were about the size of hazel nuts but in the right axilla
 there was a large hard swelling. Transfusion did not lead to improvement
 and death occurred 2 days later

On autopsy (Professor von Meyenburg Pathological Institute
 University of Zurich) the majority of the lymphatic glands were enlarged
 but especially those of the upper part of the body and showed the same
 histological picture as described before. Liver of 2 000 gm. Infiltrations
 in Glisson's capsule around the central veins and also in the parenchyma.
 The great majority of the cells were more or less round with relatively
 large nuclei and narrow cytoplasm. The fibrils between these cells were
 very scanty and delicate. A few of the large cells contained 2 nuclei and
 it was possible to observe transitions from indented to lobulated types

Spleen 1 080 gm. the trabeculae were delicate and the follicles were
 numerous. Pale round cells with lobulated and sometimes double nuclei
 were seen in the follicles and in the congested pulp with the formation of
 many reticular fibrils. A histological diagnosis of *paramyeloblastic
 leukaemia* was made

This was a typical case of primary reticulosis in which the lymphatic

was poor the liver and glands were not enlarged. Blood: red corpuscles 2.9 million, haemoglobin 49 per cent, leucocytes 720 with 32 per cent neutrophils (of which 22 per cent were staff forms), lymphocytes 56 per cent, monocytes 12 per cent, platelets 3 000. At this time no abnormal cells were found in the blood. Sternal puncture showed distinct immaturity and signs of inhibition (hypersplenism) but no abnormal cells. Serum protein 5.9 mg per cent. The BSR was 7 mm, which was surprising as there was high intermittent fever. Blood culture was negative and neither penicillin nor sulphone had any effect.

Spleen Puncture Many scanty normal lymphocytes and occasional myelocytes and erythroblasts. There were 25 per cent of large reticular tumour cells showing great variation in shape and size. As Fig 52 c shows these had a relatively large nucleus which was rounded, also often showing indentation or lobulation. The chromatin was delicate but very densely packed, especially in the older forms. The nuclei were mainly small (3-4 in number) and were not as distinct and properly demarcated as in lymphosarcoma cells. The cytoplasm was narrow and deeply basophil, sometimes containing many small vacuoles. The mitoses showed thick short chromosomes. A few tripolar mitoses (Fig 52 d) and occasional multi-nucleated cells were seen. A diagnosis of reticoid sarcoma or of reticulosis was made. (In this case spleen puncture was performed in spite of the thrombocytopenia because the bleeding time was not prolonged.)

Course After diagnosis had been made irradiation of the spleen was tried and after 4 treatments the temperature fell and in spite of the repeated irradiation the leucocytes increased somewhat and the general condition improved. Then the pyrexia recurred and the inguinal and axillary lymphatic glands increased to the size of walnuts.

Gland Puncture showed the presence of tumour cells of the same type as those in the spleen associated with almost complete absence of normal lymphocytes.

Histological examination (Professor von Albertini, Pathological Institute, University of Zurich) showed the structure of a round-celled sarcoma.

The glandular enlargement nearly disappeared without further treatment and the general improvement was so great that the patient was discharged in February 1948 when the haemoglobin was 88 per cent and the leucocytes 3 800. The spleen was now scarcely palpable (14 cm) and there were no enlarged lymphatic glands.

He remained in good health until May 1948 when there was a relapse with fever, leucopenia and swelling of the spleen and various lymphatic glands. Then a second spontaneous remission occurred and the patient was able to return to light work. In July 1948 there was a relapse and the patient was readmitted to hospital. The liver and spleen were distinctly enlarged but no lymphatic glands could be felt. The leucocytes were 400-900 but rose to 3 700 with 53 per cent of tumour cells shortly before death. No definite tumour cells were ever found in the bone marrow. Death occurred on September 6th 1948.

Autopsy (Professor von Meyenburg, Pathological Institute, University of Zurich) Splenomegaly was confirmed and many enlarged abdominal lymphatic glands were discovered. Histologically these showed severe changes but the capsule was intact. All the sinuses were narrowed and the lymphatic tissue was completely replaced by abnormal tissue in which a few scattered lymphocytes could be found. The characteristic cells

The diagnosis of the pathologist based on the excised lymph gland had pointed before (when patient was alive) to a reticulo sarcoma. The findings demonstrate the difficulties arising for the pathologist in the diagnosis of such cases and that only the close co operation between clinician and pathologist enables a distinct evaluation of such cases.

There is no doubt that this case should be included among the neoplastic proliferations of the reticulo endothelium but whether it has to be regarded as a 'retiothel' sarcoma with a leukemic blood picture, in the sense used by Apitz, is not really of much importance.

A summary of another of our cases is of interest at this point, because it shows that temporary partial remissions can occur in this neoplastic disease just as they can in the leukæmias.

Case 2 K. E. aged 25 policeman (November 1947 to September 1948 †)

The disease started with weakness and fever in November 1947 when the spleen was found to be distinctly enlarged the general condition

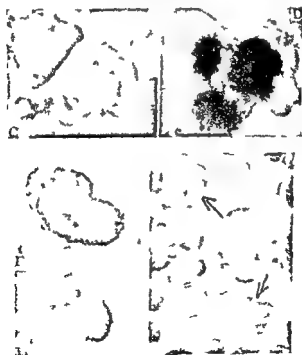


FIG 52 c f *Reticuloses* (Spleen puncture)

(c) *Case 2* male aged 25 The cells have intensely basophilic cytoplasm with compact chromatin and small indistinct nucleoli

(d) Abnormal tri polar mitoses from the same case

FIG 52 e f g *Case 6* female aged 72 Large lobulated cells with strikingly dense chromatin (e) Spleen puncture (f) Blood film (g) Histological section of the spleen

was poor the liver and glands were not enlarged. Blood: red corpuscles 7.9 million, hemoglobin 49 per cent, leucocytes 720 with 32 per cent neutrophils (of which 22 per cent were staff forms), lymphocytes 56 per cent, monocytes 12 per cent, platelets 3 000. At this time no abnormal cells were found in the blood. Sternal puncture showed distinct immaturity and signs of inhibition (hypersplenism) but no abnormal cells. Serum protein 5.9 mg per cent. The BSR was 7 mm, which was surprising as there was high intermittent fever. Blood culture was negative and neither penicillin nor sulphone had any effect.

Spleen Puncture. Many scanty normal lymphocytes and occasional myelocytes and erythroblasts. There were 25 per cent of large reticular tumour cells showing great variation in shape and size. As Fig 52 c shows these had a relatively large nucleus which was rounded, also often showing indentation or lobulation. The chromatin was delicate but very densely packed, especially in the older forms. The nucleoli were mainly small (3-4 in number) and were not as distinct and properly demarcated as in lymphosarcoma cells. The cytoplasm was narrow and deeply basophil, sometimes containing many small vacuoles. The mitoses showed thick short chromosomes. A few tripolar mitoses (Fig 52 d) and occasional multi-nucleated cells were seen. A diagnosis of reticoid sarcoma or of reticulosis was made. (In this case spleen puncture was performed in spite of the thrombocytopenia because the bleeding time was not prolonged.)

Course. After diagnosis had been made irradiation of the spleen was tried and after 4 treatments the temperature fell and in spite of the repeated irradiation the leucocytes increased somewhat and the general condition improved. Then the pyrexia recurred and the inguinal and axillary lymphatic glands increased to the size of walnuts.

Gland Puncture showed the presence of tumour cells of the same type as those in the spleen associated with almost complete absence of normal lymphocytes.

Histological examination (Professor von Albertini, Pathological Institute, University of Zurich) showed the structure of a round-celled sarcoma.

The glandular enlargement nearly disappeared without further treatment and the general improvement was so great that the patient was discharged in February 1948 when the haemoglobin was 88 per cent and the leucocytes 3 800. The spleen was now scarcely palpable (14 cm) and there were no enlarged lymphatic glands.

He remained in good health until May 1948 when there was a relapse with fever, leucopenia and swelling of the spleen and various lymphatic glands. Then a second spontaneous remission occurred and the patient was able to return to light work. In July 1948 there was a relapse and the patient was readmitted to hospital. The liver and spleen were distinctly enlarged but no lymphatic glands could be felt. The leucocytes were 400-900 but rose to 3 700 with 53 per cent of tumour cells shortly before death. No definite tumour cells were ever found in the bone marrow. Death occurred on September 6th 1948.

Autopsy (Professor von Meyenburg, Pathological Institute, University of Zurich). Splenomegaly was confirmed and many enlarged abdominal lymphatic glands were discovered. Histologically these showed severe changes but the capsule was intact. All the sinuses were narrowed and the lymphatic tissue was completely replaced by abnormal tissue in which a few scattered lymphocytes could be found. The characteristic cells

were rather large, round oval or elongated elements which in places formed a network. The nuclei were round or slightly indented usually being large and varying in the amount of chromatin they contained. Very few mitoses were seen but a few cells had 2 or 3 nuclei. These elements lay in contact with a network of very delicate fibrils most of which could be stained with silver also in addition there was an increase of collagen. The arrangement of these reticulum was so diffuse that the systematic arrangements of normal lymphatic glands could no longer be detected.

Spleen The appearances were very similar to those in the lymphatic glands. The spleen tissue was replaced by the described cells and a network of reticular fibrils.

The Bone Marrow The greater part was more or less normal but contained hæmorrhages and focal infiltrations of the typical tumour cells associated with increase of reticulum fibres.

The other organs appeared normal.

Summary The febrile attacks which occur in many reticuloses were well marked while the spleen and glands were also involved. The bone marrow in the early stages was unaffected. There were two marked spontaneous remissions so that it must be assumed that in this disease as in the leukæmias there is some unknown defensive mechanism against the number of proliferating neoplastic cells although this cannot permanently inhibit their growth.

A particularly striking feature of this case was the extreme inhibition of the marrow which developed during each relapse and which was doubtless due to hypersplenism.

Histologically in the spleen glands and in small foci in the marrow there was infiltration with proliferating reticular cells together with obvious formation of reticular fibrils.

That these were definitely neoplastic elements not simply due to a reaction is shown by the gross abnormalities of structure of the characteristic cells (Fig. 52 c and d) and also by the occurrence of abnormal mitoses. The case also confirms our previous statement that when there is well marked formation of reticular fibrils there are generally no striking changes in the serum proteins.

Of course reticulo-endothelial proliferations may originate in the marrow and then it is very rarely possible to distinguish the condition from paramyeloblastic leukæmia. But this distinction can be made if the cells are excessively large or if the chromatin is very different from that of myeloblasts (e.g. Kienle's case). It is also obvious when the cells have bulky basophilic cytoplasm as in plasma cell myeloma. Kienle has recorded and illustrated a case in which the sternal marrow contained some enormous basophilic cells although none were found in the blood. At autopsy there were milium aggregations of these elements in the spleen glands and liver. In cases in which the changes are less striking exact classification is not possible if the marrow is extensively involved.

Dr. Jucker (Medical Clinic Basel under the Directorship of

Professor Staub) has kindly given me access to material from another case (Case 3) of leukæmic reticulosis in which only the liver and spleen were affected while the marrow was normal. Spleen, liver and sternal punctures were carried out during life and a complete autopsy was performed. Fig. 53 *a* is from spleen films kindly given to us by Dr Jucker. It shows large (18–35 μ) round cells with deeply basophilic vacuolated cytoplasm. There is a resemblance to myeloma cells but the nuclei are larger, denser and sometimes lobulated.

I owe Case 4 to Dr Ludin (Medical Clinic, Basel). Professor Staub. It was that of a woman of 67 with a considerable degree of



FIG. 53 (*a*). *Acute primary reticulosis of liver and spleen.* Large atypical myeloma cells in spleen puncture (Dr Jucker's case from which Professor Staub (Basel) kindly supplied spleen films.)

insidious splenomegaly, greatly increased B.S.R. (103/122) and slight inhibition (probably splenogenic) of the marrow (leucocytes 3,700). In spleen puncture there were 96 per cent of lymphoid cells, varying greatly in size and having a rather closely woven nucleus. There was a distinct resemblance to the large lymphatic reticulum cells that are found in normal gland punctures. At first we made a tentative diagnosis of aleukæmic lymphadenosis but in view of the greatly increased B.S.R. concluded that it was a reticulosis. The autopsy (Professor Werthemann, University of Basel) revealed the typical features of a reticulosis with great increase of fibrils and proliferation of reticulum cells in the spleen and glands. In places the capsule was penetrated by these elements. This case

were rather large round, oval or elongated elements which in places formed a network. The nuclei were round or slightly indented usually being large and varying in the amount of chromatin they contained. Very few mitoses were seen but a few cells had 2 or 3 nuclei. These elements lay in contact with a network of very delicate fibrils most of which could be stained with silver also in addition there was an increase of collagen. The arrangement of these reticulum was so diffuse that the systematic arrangements of normal lymphatic glands could no longer be detected.

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The repeated punctures of spleen glands and marrow in our cases have demonstrated that in the early stages of primary reticuloses the disease is not systematic (We think that this is also the case in the leucoses). The initial neoplastic dedifferentiation seems to be localised (perhaps unicentric) later giving rise to metastases especially in lymphatic tissue (also although less frequently in the marrow liver and other organs). It seems as if these cells find the most suitable living conditions in their parent tissue. It is because the pathologist only sees these cases in their terminal stage that he usually (Apitz) considers them to be examples of a system disease. Although we cannot accept this idea in most cases we would not deny the possibility of an occasional pluricentric or systematised malignant change such as Rohr has suggested in a few cases (perhaps due to a virus infection). Even so not all reticuloses are genuine system-diseases. We agree with Van der Meer and Zeldendrust (1948) that there are transitions between primary reticuloses reticulo sarcoma with localised growth the leucoses and Hodgkin's disease

We have devoted so much space to the reticuloses because they seem to be a group in which spleen and marrow puncture sometimes amplified by gland puncture are particularly useful for the gaining of an insight into the pathological process

XII SARCOMAS OF THE SPLEEN

1 LYMPHOSARCOMA

This quite commonly originates in the spleen (in 21 per cent of cases according to Sugerbaker) and then rapidly metastasises in other lymphatic tissues. Sometimes malignant change starts in a lymphatic gland while the spleen is secondarily involved. In the later stages the tumour cells may overflow into the blood giving rise to a leukæmic picture with 50 000 or more white cells. We

was the only exception of marked serum protein changes and formation of reticular fibrils at the same time

This case clearly illustrates our earlier contention that there is so close a relation between the lymphadenoses and the reticulososes that a distinction may be rather difficult

There is also a close relationship between some atypical forms of Hodgkin's disease and the reticulososes; this applies particularly to the so called Hodgkin sarcoma

Dr Buchler (Tiefenauhospital Bern) has kindly given me spleen films from an important case (Case 5). A man of 21 showed areas of rarefaction and others of sclerosis in the skeleton. There was only slight enlargement of the lymphatic glands which histologically showed only the picture of a chronic inflammation with plasma cells and many eosinophiles but no Dorothy Reed cells. In the marrow there was an excess of eosinophiles together with small groups of lipoid cells. Spleen puncture revealed the presence of up to 60 per cent of reticular tumour cells with deeply basophilic cytoplasm resembling glandular fever cells of varying stages of maturity. Their great variation in size and the numerous mitoses clearly distinguished them from these elements. An occasional cell of this type was found in the blood. There was no excess of eosinophiles in the spleen.

Although no autopsy has yet been performed it seems probable that this is a case of *primary reticulosis* in which there are resemblances to Hodgkin's disease. It seems possible that the eosinophilia in the glands and marrow as well as the pyrexia are due to a sensitisation to some protein substance set free by the proliferating tumour cells. We suggest a similar explanation for the eosinophilia and fever of Hodgkin's disease which we also regard as neoplastic (see Moeschlin and Schwarz).

A striking difference between the cells of reticulosis and those of Hodgkin's disease is the absence of the bluish violet nucleoli so characteristic of Sternberg cells. For instance in Case 6 (a woman of 72) there was persistently high fever associated with distinct splenomegaly; but no clinical diagnosis could be made. The pathologist was able to demonstrate the presence of actively proliferating large cells with distinct lobulation in the spleen, liver and lungs. His diagnosis was *atypical lymphogranuloma*.

A glance at Fig. 52 *e f g* shows that these elements are in fact large lobulated reticular cells similar to those found in spleen puncture and that they are very different from those of Hodgkin's disease. Even in the less mature forms no bluish violet nucleoli could be found. Our view is therefore that this also is an example of genuine reticulosis.

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VII SARCOMAS OF THE SPLEEN

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This quite commonly originates in the spleen (in 21 per cent of cases according to Sugerbaker) and then rapidly metastasises in other lymphatic tissues Sometimes malignant change starts in a lymphatic gland while the spleen is secondarily involved In the later stages the tumour cells may overflow into the blood giving rise to a leukæmic picture with 50 000 or more white cells We

have already discussed such a case in the section on acute lymphatic leukaemia (p 128) On the whole, lymphosarcoma differs from the relatively benign chronic lymphadenoses in its rapid and infiltrative growth

Clinically lymphosarcoma is characterised by enlarged, relatively soft glands sometimes by splenomegaly and usually by pyrexial



FIG. 53 b c. *Lymphosarcoma of the spleen*

(b) Spleen puncture. Intensely basophilic cells. Arrangement of the chromatin in masses is characteristic of the more mature types. The large distinct nucleus is unlike that seen in the cells of chronic lymphatic leukaemia.

(c) Phase contrast picture. The large dark permanent nucleoli are very characteristic and are very different from the small numerous nucleoli found in lymphoblastoma and lymphatic leukaemia. (Zeiss phase contrast microscope 1,000)

episodes. In 2 cases there was extreme leucopenia probably due to splenopathic inhibition of the marrow. The pyrexia is probably due to sensitisation to protein substances liberated by degenerating tumour cells. This is similar to the state of affairs in association with other tumours and with Hodgkin's disease.

Structure of Lymphosarcoma Cells. There is a general resemblance to the younger forms of lymphatic cells but the lymphosarcoma cells differ from them by their great variation in shape and

size and especially by the presence of large vesicular nucleoli (Fig 53 b c)

The nucleus is roundish or oval often slightly indented and of very variable size but usually larger than that of a normal lymphocyte. The finely granular chromatin is reminiscent of that of the lymphoblasts but is usually arranged in rather coarser masses while in the older forms it is denser and arranged in more sharply demarcated masses. Some cases show rosette forms of the nucleus (Fig 53 c). The cytoplasm is usually narrow but varies from cell to cell it may be fairly bulky and is always intensely basophilic. *The most characteristic features are the nucleoli.* As is shown in Fig 53 b there is usually a very large rather pale nucleolus which is separated from the rest of the chromatin by a distinct membrane. Occasionally there are two or three large nucleoli but more often there is one large one and several smaller ones. Mitoses are numerous and the chromosomes are narrow lying at an acute angle.

Phase Contrast Microscopy. As we were the first to point out (Moeschlin (23 24) the large nucleoli are particularly well seen being strikingly dark. This is a useful point of distinction from the smaller and paler nucleoli of young lymphocytes and from the cells of lymphoblastoma (Fig 53 e). In doubtful cases fresh preparations should always be examined with the phase contrast microscope.

The typical characters of lymphosarcoma cells (variation in size narrow basophilic cytoplasm large nucleoli and numerous mitoses) can more readily be appreciated in gland puncture (Tischendorf 1 3 Stahel 1) and spleen puncture than in sections where it may be impossible to distinguish these cells from other somewhat similar ones.

X ray. Strunge has shown that structural changes can be found in lymphosarcoma cells as early as 5 hours after *deep X ray* therapy but that these are no longer detectable after 48 hours when active cell division again starts. *This observation emphasises the need for daily treatment for long periods without any prolonged intermissions* a fact often mentioned by the radio therapists.

Nitrogen Mustard. This treatment is followed by rapid decrease in size of the spleen and glands. Puncture 24 hours after intra venous injection of 2 mg showed evidence of an intense cytotoxic action viz rapid disintegration of pyknotic cells the presence of many macrophages filled with cell debris and virtual cessation of mitosis. (Signs of damage to the process of cell division could often be seen e.g. clumping of the chromosomes etc.)

2 FOLLICULAR LYMPHOBLASTOMA

(Brill Symmers Disease)

This disease is probably a neoplastic proliferation of the cells of the germ centres i.e. of those elements we have called large lymphatic reticulum cells.

There is gradual enlargement of all the lymphatic glands the

masses being strikingly soft. Histologically the appearances are those of enormous enlargement of the germ centres. The course is long extending over several years while in the early stages, the general condition of the patient remains unimpaired (Salm).

That these tumours are more related to the reticular cells than to the lymphocytes is made evident by the existence of rare cases



FIG. 53 *d & e* Lymphoblastoma folliculare (Brill-Symmers disease)

(d) Spleen puncture. Numerous large polymorphic cells with indistinct but rather delicate chromatin somewhat resembling large lymphatic reticulum cells together with delicate epithelioid cells which mainly lie in groups of two or three.

(e) Phase contrast picture. The variations between these cells and those of lymphosarcoma are more striking by this method. The numerous indentations of the nuclei sometimes in a rosette form and the numerous small nucleoli are clearly seen. On the left there are two epithelioid cells.

with a dysproteinemia (Rossier-Spuhler) showing the same changes as seen in myeloma and primary malignant reticulosis.

The spleen is almost invariably involved (Mayer 1939). Thus Baggenstoss (1940) found splenomegaly in 61 per cent of cases. Stahel (1948) was the first to report on the appearances found by gland puncture in which he saw not only increase of germ centre cells but also occasional giant cells with 2 or 3 nuclei.

There are no records of spleen puncture but owing to the

kindness of Professor Schinz (Röntgen Institute University of Zurich) we have been able to investigate a man of 70 in whom the diagnosis of this disease had been made by excision of a gland. The spleen was enlarged (25 cm) and we were able to follow the effects of treatment with nitrogen mustard.

Spleen Puncture A few normal lymphocytes, granulocytes and plasma cells were seen but up to 84 per cent of the cells resembled those of the germ centres (our large lymphatic reticulum cells).

The nuclei are rather indistinct and consist of fairly dense chromatin composed of delicate connections. There are 2-4 pale but small nucleoli (Fig 53 d). Many of the nuclei show delicate indentations and lobulation while a few present a rosette arrangement. The cytoplasm is narrow and intensely basophilic, the edge being irregular. Mitoses are fairly numerous, the chromosomes being delicate and at an acute angle.

In addition to the more obvious tumour cells there are smaller types and occasional giant-cells with 2-3 nuclei and wide cytoplasm.

The nuclei have a rather coarse meshwork and their structure is somewhat reminiscent of the epithelioid cells of tuberculosis and of the Dorothy Reed cells of Hodgkin's disease. But unlike the latter they rarely contain bluish nucleoli (Fig 53 d).

The cells exactly resemble the giant-cells described in gland puncture by Stahel and are the same as those described by Kellert and Terplan in sections. Presumably these malformations are due to failure of the cytoplasm to divide when the nucleus which is becoming pyknotic has done so.

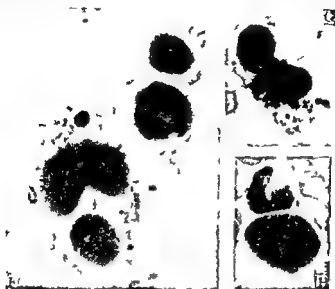
Gland Puncture The appearances were very like those in the spleen except that there were practically no normal lymphocytes, plasma cells or granulocytes.

Phase Contrast Microscopy Elsewhere (Moeschlin (24)) we have pointed out that this method of examination permits one to distinguish between these cells and normal ones as well as the abnormal elements of reticulosis, lymphosarcoma and lymphadenosis. In stained films confusion between these last two is almost inevitable. *Phase microscopy reveals the cells of lymphoblastoma as having small dark nucleoli unlike the larger but equally dark ones of lymphosarcoma cells.* They are distinguished from the lymphocytes of lymphadenosis by their great tendency to indentation and narrowings of the nuclei which may lead to the formation of rosettes (Fig 53 e) which are rare in other diseases of glands except in lymphosarcoma.

Treatment with nitrogen mustard is followed by rapid decrease in size of glands and spleen together with gradual diminution of abnormal cells and reappearance of normal lymphocytes. Many necrotic cells are present but one of the most striking features is reduction in the number of mitoses as well as the occurrence of atypical ones e.g. with condensed or swollen chromosomes.

3 MELANOSARCOMA

The presence of metastases in other organs (skin liver etc) and perhaps the black colour of the tumours usually makes the diagnosis obvious. The appearance of the typical pigmented cells

FIG 53 f g *Melanoma sarcoma*

(f) A group of epithelioid cells some containing melanin others not pigmented

(g) Cells containing much melanin

FIG 53 h *Retiothel sarcoma* (Spleen puncture)

These cells show distinct blue or bluish violet nucleoli and can be confused with Sternberg cells. The splenogram does not present the chronic inflammatory character that is found in Hodgkin's disease

is seen in Fig 53 f g. Difficulty can arise if as is not uncommon the malignant cells contain no melanin

4 OTHER FORMS OF SARCOMAS

Unlike lymphosarcoma and lymphoblastoma in which the characteristic cells show some resemblance to lymphatic elements the cells of the undifferentiated sarcomas have a very different nuclear structure. We prefer the term undifferentiated sarcoma to those commonly used by pathologists viz mixed celled sarcoma and giant celled sarcoma

We have records of 4 cases in which the growth probably did not arise primarily in the spleen but grew into it from the retroperitoneal tissues. In one case spleen puncture revealed an ordinary chronic inflammatory picture. At autopsy focal deposits of tumour were found but these had obviously been missed by the needle. In the other 3 cases tumour cells were found

Retothel Sarcoma A man of 22 with a large firm spleen which moved with respiration. Leucocytoses of 12 000 with some leftward shift but no abnormal cells in blood films. Hæmoglobin 84 per cent. B S R 30 mm.

In spleen puncture scattered nuclei were found. These were large and were composed of delicate but closely arranged chromatin with one or two distinct bluish nucleoli. The cytoplasm was narrow and stained slightly blue (Fig 53 h).

The presence of bluish nucleoli suggested a diagnosis of retothel sarcoma or of Hodgkin's disease but the absence of plasma cytoid reticulum cells and eosinophiles was against the latter possibility. Operation revealed a large retroperitoneal retothel sarcoma which had pushed the enlarged spleen forward.

The presence of bluish nucleoli which has also been reported by Ludin seems to be a valuable feature in distinguishing the retothel sarcomas from the true reticuloses in which the puncture may in other respects be very similar.

Undifferentiated Sarcoma In this very undifferentiated type (mixed celled and giant celled) of growth spleen puncture shows large groups of cells the cytoplasmic boundaries of which are not always recognisable. Such a syncytial arrangement is shown in Fig 54 a b. This is very different from the condition in reticuloses lymphosarcoma and lymphoblastoma in all of which only single cells or at the most small groups (in reticuloses) can be found.

The chromatin is closer and smoother than in lymphosarcoma, and there is no tendency to an arrangement in fields such as is characteristic of lymphosarcoma. Nucleoli are small and are sometimes difficult to detect. Nuclear indentation may be seen but it never reaches the degree of lobulation found in some reticuloses.

If these features of big syncytial arrangements with numerous mitosis can be found the diagnosis of sarcoma can be made but if only isolated cells are present it may be impossible.

Fig 54 a m from spleen puncture on a woman of 62 who was admitted to the Clinic with high septic fever. The B S R was 54 mm, hæmoglobin 60 per cent and a leucocytoses of 13 900 with distinct shift to the left. There was an enormous hard tumour in the left upper abdomen.

In spleen puncture there were a few blood cells and large syncytia in which the individual elements showed much variation in size. The nuclei which were rounded were composed of closely woven but delicate chromatin. The very narrow cytoplasm was greyish violet in colour. The nucleoli were small and could not be recognised in all the cells.

Autopsy revealed a large retroperitoneal sarcoma which had infiltrated the otherwise normal spleen.

The third case was that of a woman of 21 in whom great spleno

3 MELANOSARCOMA

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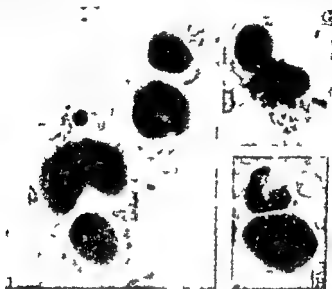


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Unlike lymphosarcoma and lymphoblastoma in which the characteristic cells show some resemblance to lymphatic elements the cells of the undifferentiated sarcomas have a very different nuclear structure. We prefer the term undifferentiated sarcoma to those commonly used by pathologists viz mixed celled sarcoma and giant celled sarcoma

We have records of 4 cases in which the growth probably did not arise primarily in the spleen but grew into it from the retroperitoneal tissues. In one case spleen puncture revealed an ordinary chronic inflammatory picture. At autopsy focal deposits of tumour were found but these had obviously been missed by the needle. In the other 3 cases tumour cells were found

Retothel Sarcoma : A man of 22 with a large firm spleen which moved with respiration. Leucocytoses of 12 000 with some leftward shift but no abnormal cells in blood films. Hæmoglobin 84 per cent. B S R 30 mm.

In spleen puncture scattered nuclei were found. These were large and were composed of delicate but closely arranged chromatin with one or two distinct bluish nucleoli. The cytoplasm was narrow and stained slightly blue (Fig 53 *h*).

The presence of bluish nucleoli suggested a diagnosis of retothel sarcoma or of Hodgkin's disease, but the absence of plasma cytoid reticulum cells and eosinophiles was against the latter possibility. Operation revealed a large retroperitoneal retothel sarcoma which had pushed the enlarged spleen forward.

The presence of bluish nucleoli which has also been reported by Ludin seems to be a valuable feature in distinguishing the retothel sarcomas from the true reticuloses in which the puncture may in other respects be very similar.

Undifferentiated Sarcoma In this very undifferentiated type (mixed celled and giant celled) of growth spleen puncture shows large groups of cells the cytoplasmic boundaries of which are not always recognisable. Such a syncytial arrangement is shown in Fig 54 *a b*. This is very different from the condition in reticuloses lymphosarcoma and lymphoblastoma in all of which only single cells or at the most small groups (in reticuloses) can be found.

The chromatin is closer and smoother than in lymphosarcoma and there is no tendency to an arrangement in fields such as is characteristic of lymphosarcoma. Nucleoli are small and are sometimes difficult to detect. Nuclear indentation may be seen but it never reaches the degree of lobulation found in some reticuloses.

If these features of big syncytial arrangements with numerous mitosis can be found the diagnosis of sarcoma can be made but if only isolated cells are present it may be impossible.

Fig 54 *a* is from spleen puncture on a woman of 62 who was admitted to the Clinic with high septic fever. The B S R was 54 mm. hæmoglobin 60 per cent and a leucocytoses of 13 900 with distinct shift to the left. There was an enormous hard tumour in the left upper abdomen.

In spleen puncture there were a few blood cells and large syncytia in which the individual elements showed much variation in size. The nuclei which were rounded were composed of closely woven but delicate chromatin. The very narrow cytoplasm was greyish violet in colour. The nucleoli were small and could not be recognised in all the cells.

Autopsy revealed a large retroperitoneal sarcoma which had infiltrated the otherwise normal spleen.

The third case was that of a woman of 21 in whom great spleno

megaly had led to a suspicion of Hodgkin's disease. Spleen puncture showed the typical syncytia, no normal spleen cells being found. Here again the undifferentiated structure of the nuclei was a striking feature (Fig. 54 b).

It must be mentioned that the discovery of such groups of cells

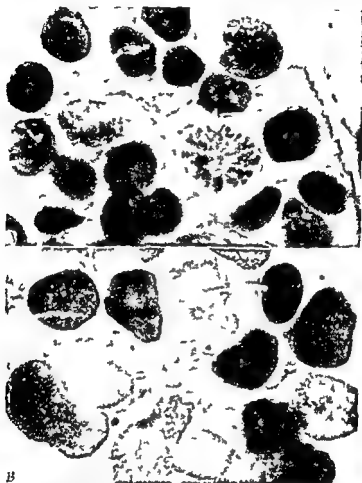


FIG. 54 a b Polymorphic cell and giant celled sarcomas of the spleen. The large groups of cells (syncytia) in which no distinct cytoplasmic outlines can be detected is characteristic and is strikingly different from the condition in reticulosus lymphosarcoma and lymphoblastoma (a) Aged 62 (b) Aged 21

should also lead one to consider the possibility of hypernephroma and even of a pancreatic tumour.

Other malignant tumours of the spleen are extremely uncommon (Lubarsch). Walther had shown that the rarity of metastases in the spleen is not due to a special defensive power of the reticulo-endothelium but only to the peculiar arrangement of the circulation which tends to prevent entry of neoplastic cells.

XIII CARCINOMAS AND CYSTS

In spite of the most careful clinical examination it is possible to mistake a mass in the left hypochondrium for an enlarged spleen when it is in fact a tumour (see Naegeli (2)). Of course spleen puncture will demonstrate this. Thus in one of our cases puncture of what seemed to be an enlarged spleen showed typical tumour

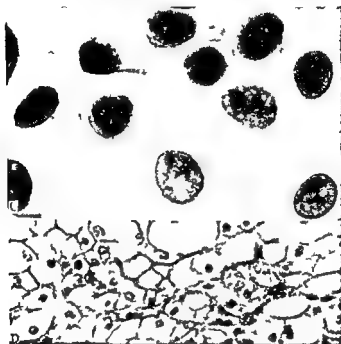


FIG 54 c d *Hypernephroma*

(c) Film from puncture (A tumour was mistaken for an enlarged spleen). The typical appearance is that of groups of cells with rather bulky foamy and round dense nuclei which show considerable variations in size.
(d) Histological section of the same puncture material

cells in films (Fig 54 c d). This was later shown to be a hypernephroma. Weil has reported a similar case. I am grateful to Dr Vegh (of Szeged) for a further example.

Structure of Hypernephroma Cells These are strikingly large but variable ($15-80\ \mu$). The round nucleus is relatively small ($10-24\ \mu$) and is composed of dense chromatin in which a small nucleolus can sometimes be seen. The cytoplasm sometimes resembles a honeycomb but is usually a homogeneous pale violet or reddish violet colour. Rarely some blackish pigment is present in the cells. Cells with 2 or 3 nuclei may also occur. The typical features are the occurrence of the cells in fairly large aggregations and the great variation in size of their nuclei and cytoplasm (Fig 54 c d). The latter feature also distinguishes them

from smaller cells of the pulmonary alveoli to which they have a distinct resemblance (Fig. 22 d)

In one case we found undifferentiated carcinoma cells and autopsy showed that the cells were divided from a carcinoma of the pancreas

Cysts A woman of 35 had a large firm tumour in the left upper abdomen which radiographically, appeared to be an enlarged spleen. Radiotherapy produced no improvement indeed there was slow but progressive increase in size. Puncture was therefore performed and sero sanguineous fluid, under pressure was obtained. No microscopic signs of hydatid were found and operation (Professor Clairmont) revealed an enormous pancreatic cyst. If therefore, spleen puncture results in such a fluid, it is necessary to investigate its diastase content which we unfortunately did not do.

Weil (1) recorded 2 cases in which a sero sanguineous fluid was obtained. One proved to be a dermoid cyst of the spleen while the other was a cystic angioma of the left lobe of the liver. Other causes of enlargement of this lobe must be borne in mind. Puncture will show the presence of liver cells with bulky finely granular cytoplasm and a small round nucleus (Stahel (3)).

E DEGENERATIVE CONDITIONS OF THE SPLEEN

XIV AMYLOIDOSIS

We have punctured one case of almost certain amyloidosis (congo red test positive) but the dense consistency of the organ prevented us from obtaining any spleen substance. In such cases sections would probably establish the diagnosis but examination of films would not be likely to do so.

F STORAGE DISEASES OF THE SPLEEN

XV GAUCHER'S DISEASE

Spleen puncture has often been used for diagnosis e.g., by Sokolowski (1932) Potter and McRae (1933) Pick (1933) Merklen (1933) Lowinger (1935) and Fppinger (1939). Since the discovery of Gaucher cells by sternal puncture (Lowinger Klum Rohr etc) spleen puncture has become of less importance. If as is sometimes the case sternal puncture fails to reveal the characteristic cells (Pittaluga and Rof Barchasch and Gurin Renard) spleen puncture is indicated.

We have records of 4 cases (including 2 brothers) in which these cells were found (2 of the cases were under Professor Löffler).

The cells are very large (30 or 40 μ up to 60 μ) round or oval

elements with bulky cytoplasm and one or more nuclei lying near the edge (Eppinger *et al*) (Fig 55 a)

The reddish or violet cytoplasm is not sharply demarcated from the background and stained with Giemsa has an appearance resembling crinkled tissue paper. This results from the network that remains after the lipoid (kerasin) has been extracted by alcohol. The older cells are almost colourless and are often seen as blank spaces between more obvious cellular elements while younger cells are distinctly basophilic. The small round nucleus usually lies at the periphery of the cell the chromatin being arranged closely but in a coarse network. up to four

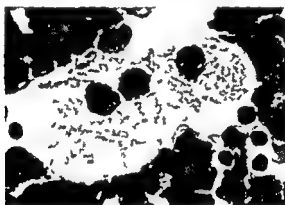


FIG 55 a Gaucher cells, some with 2 nuclei

nuclei may be present in one cell. Only di Guglielmo (3) has recorded the presence of Gaucher cells in the peripheral blood.

Unlike Niemann Pick cells (Fig 55 b) Gaucher cells do not contain distinct cytoplasmic vacuoles.

TABLE 31
Gaucher's Disease

	1	2		1	2
<i>Hæmogram</i>			<i>Plasmacyt retic cells</i>	0.6	—
Hæmoglobin ()	82	—	<i>Pulp cells</i>	0.2	—
Red corpuscles (in mill)	3 984	—	<i>Erythroblasts</i>	0.7	—
Thrombocytes	47 800	—	<i>Myelocytes</i>	1.6	1.0
Leucocytes	5 400	—	<i>Neutrophiles staff</i>	14.6	2.8
Myelocytes	14	—	<i>Neutrophiles segmented</i>	26.8	4.6
Neutrophiles staff	71	—	<i>Eosinophiles</i>	2.6	—
Neutrophiles segmented	69	—	<i>Basophiles</i>	0.5	—
Eosinophiles	1	—	<i>Monocytes</i>	5.8	—
Monocytes	3	—	<i>Lymphocytes young</i>	2.2	1.4
Lymphocytes	17	—	<i>Lymphocytes old</i>	41.8	81.0
Plasma cells	—	—	<i>Lymphocytes total</i>	44.0	82.4
			<i>Plasma cells</i>	0.2	—
<i>Sp enog am</i>			<i>Gaucher cells, young</i>	0.3	0.4
<i>Macrophages</i>	—	—	<i>Gaucher cells old</i>	2.1	8.8
			<i>Gaucher cells total</i>	2.4	9.2

The presence of many such elements in the spleen puncture points distinctly to Gaucher's disease. In Section I we have mentioned the occurrence of a few large lipophages in spleen puncture in inflammatory conditions (Fig 20). There is a special difference in size—the lipophages of chronic inflammation were never more than 30μ and we have never seen more than 8 of them on one slide. In Gaucher's disease instead there may be 24–92 per cent of typical (larger) Gaucher cells (Table 31).

XVI NIEMANN PICK DISEASE

In children the rare Niemann Pick disease must be considered. Here, phosphatides are laid down in the cells, and death occurs quite early. Professor Willi (Sauglingsheim, Zurich) kindly allowed

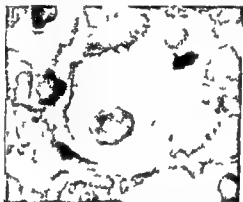


FIG 55 b Niemann Pick disease

The typical appearances of these storage cells is that of a distinct fine network in the cytoplasm as compared with the less distinct structure of Gaucher cells (Male aged $1\frac{1}{2}$ years the patient of Dr Willi of Zurich)

us to study a case in a child of $1\frac{1}{2}$ years. Spleen puncture showed normal cells together with 28 per cent of storage cells similar to those seen in sections by Pick and in sternal marrow smears by Esser, Kienle and Bessis.

Structure. Although the cells are very like Gaucher cells they are less homogeneous in stained films. The cytoplasm is completely filled with distinct small vacuoles (Fig 55 b) so that there is a close resemblance to fat macrophages (Fig 16 a-d) although of course the latter are always scanty while the former occur in groups and are more numerous.

A peculiar form of xanthomatosis of the spleen occurring in a child aged 6 years with deposits of lecithin and cephalin has been described by Dreyfuss and Fishberg.

In the Schuller-Christian disease there is deposition of sterols

(and raised blood cholesterol) but the spleen is not usually enlarged (Eppinger (2)). Quattrin has described the typical cells in bone puncture and found that they differ from Gaucher cells by the reticular structure of their cytoplasm.

Glycogen storage disease is rarely accompanied by splenomegaly. *Hamochromatosis* has been mentioned in the section on hepatic cirrhosis.

G SPLENOMEGALY OF UNCERTAIN ÆTIOLOGY

Here we shall first discuss persistent eosinophilia with splenomegaly and then two personal observations on what may well be previously unknown maladies.

Persistent Eosinophilia with Splenomegaly. A number of authors have described cases with great eosinophilia accompanied by splenic enlargement (Cremer 1939, Tischendorf 1940, Meyer 1942). We have not seen a case but the literature leads us to assume that this is only a *syndrome* in which are included familial eosinophilia (Stewart Atmar), so called eosinophil leukæmias (Thomsen and Plum), some unrecognised cases of Hodgkin's disease, parasitic infestations and some chronic infective allergic conditions such as Löffler's eosinophil mural endocarditis. Doubtless combined spleen and marrow punctures will gradually clarify the position. In one case Heilmeyer demonstrated the leukæmic nature of an eosinophilia by inducing a return to normal numbers after urethane. It is possible that in cases of non leukæmic origin the number of lymphocytes is not reduced as much as in spleens of leukæmic origin which in the first case also contains probably many immature eosinophiles.

Splenomegaly with Intense Lipæmia. We have records of one such case in which even prolonged clinical observation did not succeed in explaining the condition.

D. A. aged 28 complained of fatigue and lumbar pain in the autumn of 1941. He was admitted to hospital on February 15th 1942.

General condition poor, skin pale, no enlargement of glands or liver. Spleen distinctly palpable, rather soft and slightly tender on pressure. Urine, no red corpuscles or doubly refractile substances, a trace of albumin. Esbach 11.25. Blood pressure 130/100. Renal function tests normal. B. S. R. 6 mm. W. R. and Brucella agglutination test negative, serum protein normal 6.5, bilirubin 0.58, sodium chloride 602, calcium and serum phosphatase normal. Takata negative. Weltmann prolonged to 0.15. Persistent lipæmia (macroscopically visible). Fatty acids greatly increased 1,600–3,600 mg per cent, cholesterol high 324, esters 48, prothrombin reduced to 70 per cent but rose to 100 per cent after Synkavit. B. M. R. +20 per cent. No radiographic changes in bones.

Blood. Hæmoglobin 110 per cent, red corpuscles 5.04 million.

reticulocytes 1.6 per cent leucocytes 6 900 metamyelocytes $\frac{1}{2}$ per cent staffs $13\frac{1}{2}$ per cent segmented $59\frac{1}{2}$ per cent eosinophiles $1\frac{1}{2}$ per cent, basophiles $\frac{1}{2}$ per cent monocytes $2\frac{1}{2}$ per cent lymphocytes 22 per cent thrombocytes 113 000 without structural abnormalities Osmotic resistance of red corpuscles normal Myelogram normal Spleen puncture see Table 32

Course The lipæmia remained unchanged even on a fat poor diet while a high carbohydrate intake plus insulin also had no effect No increase of faecal fat Discharged from hospital at the end of 3 months with the splenomegaly, lipæmia and slight albuminuria unchanged

In this case a man of 28 had a distinctly enlarged spleen, lipæmia (fatty acids between 1 600 and 3 600 mg per cent), cholesterinaemia (324 mg per cent) persistent but slight albuminuria and no signs of renal damage All other investigations proved negative, so that a lipid nephrosis can be excluded Spleen puncture revealed an almost normal splenogram with a few mature myelocytes (1 per cent) and an occasional erythroblast (0.1 per cent) *No lipophages were found even after very painstaking search of spleen films* This is strong evidence against any type of xanthomatosis Perhaps we are dealing with a previously unknown disturbance of fat metabolism in which the splenomegaly was purely fortuitous

TABLE 32

<i>Hæmogram</i>		<i>Pulp cells</i>	0.2
Hæmoglobin (°)	110	Erythroblasts	0.1
Red corpuscles (in millions)	5 040	Myelocytes immature	0.1
Thrombocytes	$22\frac{1}{2}$ / μ	Myelocytes half mature	0.2
	113 000	Myelocytes mature	0.1
Leucocytes	6 900	Metamyelocytes	0.6
Metamyelocytes	$\frac{1}{2}$	Total myelocytes	1.0
Neutrophiles staff	$13\frac{1}{2}$	Neutrophiles staff	6.2
Neutrophiles segmented	$59\frac{1}{2}$	Neutrophiles segmented	24.5
Eosinophiles	$1\frac{1}{2}$	Eosinophiles	1.5
Basophiles	$\frac{1}{2}$	Basophiles	0.3
Monocytes	$2\frac{1}{2}$	Monocytes	2.4
Lymphocytes	22	Lymphoblasts	0.2
		Lymphocytes young	6.6
<i>Splenogram</i>		Lymphocytes mid	55.6
Plasmacyt retic cells	1.1	Lymphocytes total	62.4
Tissue mast cells	+	Plasma cells	0.3

Harslof (1948) has described a form of familial hepato splenomegaly accompanied by hyperlipæmia colitis hæmorrhagic diathesis (thrombocytopenia) and endocrine disturbance Tannhauser (1937) had previously recorded this disease There is enormous increase of neutral fat in the serum and a moderate increase of cholesterol and lipoids In spite of the absence of enlargement of the liver colitis and endocrine disturbances it is possible that our case is of the same type

SPLENOMEGALY WITH LYMPHOCYTOSIS (POSSIBLY OF CENTRAL ORIGIN)

We have already described a case (p 91) in which a patient with a pituitary tumour had splenomegaly and 18 000 lymphocytes per c mm. We regarded this probably as a lymphatic reaction of central origin because an infective or leukæmic origin could be excluded. Perhaps there was a temporary decrease of the adrenotropic hormone of the pituitary because other signs of pituitary deficiency were also present. This would be in conformity with the experimental findings of Dougherty and White on the regulation of lymphocytopoiesis by this hormone (see our chapter on the lymphocytes p 42).

reticulocytes 1.6 per cent leucocytes 6,900 metamyelocytes $\frac{1}{2}$ per cent staffs $13\frac{1}{2}$ per cent segmented $59\frac{1}{2}$ per cent eosinophiles $1\frac{1}{2}$ per cent basophiles $\frac{1}{2}$ per cent monocytes $2\frac{1}{2}$ per cent lymphocytes 22 per cent thrombocytes 113,000 without structural abnormalities Osmotic resistance of red corpuscles normal Myelogram, normal Spleen puncture see Table 32

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	113,000	Myelocytes mature	0.1
Leucocytes	6,900	Metamyelocytes	0.6
Metamyelocytes	$\frac{1}{2}$	Total myelocytes	1.0
Neutrophiles staff	$13\frac{1}{2}$	Neutrophiles staff	6.2
Neutrophiles segmented	$59\frac{1}{2}$	Neutrophiles segmented	24.5
Eosinophiles	$1\frac{1}{2}$	Eosinophiles	1.5
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another if there is not too much admixture of blood which can be avoided by very short aspiration. At least 1 000 cells should be examined.

For diagnostic purposes it is not usually necessary to determine an exact splenogram. Careful examination of films will give a sufficiently accurate idea of the distribution of the cells and of the presence of abnormal ones. Of course serial splenograms in leukaemias especially if myelograms are available are specially instructive. If there is distinct increase of lymphocytes a full count should be done because it may be the only way to show that there is a aleukemic form of lymphatic leukaemia restricted to the spleen.

Mitosis These are strikingly rare in lymphocytes and we have here given the first quantitative estimations ever published. Thus a spleen removed by operation in a case with lymphatic hyperplasia showed only 2 mitoses of lymphoblasts in 50 000 lymphocytes. The mitotic index of lymphocytes is thus 50 times less than that of the granulocytes. We have therefore drawn the inference that the life span of lymphocytes as compared with that of granulocytes must be fairly long.

In *lymphatic leukaemia* with many lymphoblasts the mitoses may be increased to 15 or 30 times the normal but this does not occur in all cases. In young lymphocytes mitoses are so rare that we have only seen two. The idea of amitotic division is not accepted and the evidence against its occurrence is presented in some detail.

In *Section II* the characters of spleen puncture fluid in various types of splenomegaly are described.

In *cirrhosis of the liver* and in spleno portal thrombosis the splenogram is practically normal even although the spleen may be greatly enlarged. It is likely however to be unusually bloody. In cirrhosis we did find increase of plasmacytoid reticulum cells in both the spleen and the marrow. In cases of inflammatory cirrhosis there were signs of extramedullary blood formation in the spleen even a few megakaryocytes being found.

A fairly normal picture was found in *hemolytic anemias* but as a result of the increased blood destruction haemosiderin-containing macrophages were plentiful while some erythroblasts were found during periods of exacerbation. We never observed any signs of phagocytosis of red corpuscles.

In *pernicious anemia* megaloblasts are plentiful in the spleen an indication that in the absence of haemopoietic factor even the reticulum cells of this organ give rise to these abnormal elements. Probably the megaloblasts found in the peripheral blood are derived from such extramedullary foci.

We draw a distinction between an *acute inflammatory* type of spleen puncture and a *chronic inflammatory* one. In the former the lymphocytes are reduced while neutrophils including staff cells

SUMMARY

Our hæmatological and differential diagnostic descriptions are based on study of over 300 spleen punctures

With proper technique spleen puncture can now be considered as being devoid of danger, if only enlarged spleens are submitted to the procedure and if it is never carried out in the presence of a hæmorrhagic diathesis an acute septic spleen or on comatose patients. We would emphasise as do Nagy and Weil, that spleen puncture must only be performed during full inspiration

The *technique* had been modified so that penetration of the spleen is not more than 1½–2 cm. This has been done by using the needle with which the local anæsthetic is given to determine the depth at which the spleen lies both because there is pain when it reaches the peritoneum and also because its end can eventually be felt rubbing against the capsule of the organ. Then the guard on the spleen puncture needle is fixed 1½ cm deeper than this. Using this technique we have never experienced any complication

The *material obtained* is usually 1–3 drops of rather bloody fluid in the needle and a few fragments of tissue. It contains 60–80 per cent of lymphocytes together with some granulocytes and reticulo-endothelial cells which are mainly derived from the red pulp i.e. the sinuses which are of course not connected directly with the general circulation. We would call special attention to the element we have named *pulp cell* which is probably identical with the sinus endothelium of the histologists. As it is not found in gland or marrow punctures it can be regarded as characteristic of the spleen. Other important elements are the typical plasmacytoid reticulum cells (identical with those of the marrow) and large lymphatic plasma cells (of the same type as those seen in gland puncture). We regard these two forms as having different origins. Thus there are distinct differences of structure while in cirrhosis of the liver there is increase of plasmacytoid reticulum cells in the spleen and marrow without any increase of the lymphatic type. And conversely in epidemic hepatitis there is increase of lymphatic plasma cells (and many mitoses) in the spleen while marrow puncture fails to reveal an increase of plasmacytoid reticulum cells (Landolt)

We have also called attention to another type of lymphatic element found in spleen puncture viz the large lymphatic reticulum cell. This is also found in gland punctures and is probably identical with the germ centre cells seen in sections

Differential counts (splenograms) can be compared with one

In cases of the *Still Chauffard Feltz* syndrome there was some times great increase of plasmacytoid reticulum cells and occasionally of eosinophiles. Reference is also made to the occurrence of cyclic agranulocytosis in this malady.

In malaria the presence of macrophages containing melanin may allow a diagnosis to be made in cases of persistent splenomegaly even years after infection.

The findings of the causative parasites in spleen films are of course very important in *kala azar*.

We have here given the first description of tuberculous epithelioid cells in spleen puncture films. This finding is specially important in those cases of chronic splenic tuberculosis which can easily be confused with Hodgkin's disease and other causes of splenic enlargement.

In 5 out of 7 cases of *brucellosis* we found large epithelioid cells in spleen films. This is the first description of these elements in films although they had previously been seen in sections by Löffler and Albertini. In one case with negative agglutination tests the diagnosis was made by this method.

We have been able to confirm the observations of other workers on the presence of Dorothy Reed cells in spleen films from *Hodgkin's disease* and we have been able to make a diagnosis of the not rarely encountered hepatolienal form by this method when all other means had failed. This also applies to cases in which excision or puncture of glands is impossible.

Megakaryocytes can be found in spleen films from cases of toxic thrombocytopenia as well as in chronic inflammatory splenomegalies. They are rarely absent in chronic myelocytic leucoses.

Lymphatic leucoses are characterised by an almost purely lymphocytic picture (92-99 per cent). This permits the diagnosis of aleukæmic lymphadenosis which is confined to the spleen. Often but not invariably the lymphatic cells vary greatly in size while lymphoblasts and mitoses may also be prominent.

Acute lymphatic leucosis is discussed in the light of an illustrative case and the relationship of this condition to lymphosarcoma is stressed. Further the close relation of lymphadenosis and reticulosis is affirmed while those aleukæmic cases in which the BSR is greatly increased are assigned to the latter category.

In chronic myeloid leucoses the splenogram is mainly myelocytic (20-60 per cent) with 3-35 per cent of erythroblasts. If staff and segmented forms exceed the myelocytes the prognosis is relatively good whereas if the latter predominate it can be inferred that the disease is of longer (some years) duration and that neither the response to treatment nor the expectation of life will be as good. Usually the myeloblasts form 0.5-1.5 per cent of the cells, higher numbers are a bad sign. All our cases in which

and myelocytes are increased as are also the pulp cells and macrophages while an occasional erythroblast may be seen

In chronic inflammatory type, in addition to these changes the monocytes plasmacytoid reticulum cells (1-10 per cent) and some times erythroblasts (0.5-10 per cent) are distinctly increased

Hæmopoietic foci in the spleen are thus usual in inflammatory splenomegalies and cells from these foci may enter the blood in sufficient numbers to arouse together with the splenomegalia and leukocytosis a suspicion of early chronic myeloid leukæmia. Spleen puncture enables the diagnosis to be settled. In leukæmia, the myelocytes are between 20 and 60 per cent whereas in chronic inflammatory splenomegaly they do not rise above 1-5 per cent. Even when myeloid metaplasia in *osteosclerosis* and *myelofibrosis* is so intense that myelocytes are numerous in spleen films (23 per cent in one of our cases) the persistence of large numbers of lymphocytes (50-60 per cent) distinguishes the condition clearly from myeloid leukæmia.

The *lymphatic reactions* that occur in infectious mononucleosis and in epidemic hepatitis are discussed in some detail.

In *glandular fever* at the height of the disease spleen puncture reveals a considerable excess of reticulo endothelial cells some in mitosis with transitions between them and the typical glandular fever cells. Clearly then, these elements arise in the spleen whence they pass into the blood. This would explain the blood changes that are found in those splenomegalic cases in which there is no enlargement of lymphatic glands. Probably other lymphotropic viruses can evoke similar responses.

In *Q fever* we found a definite lymphatic reaction with increase of young lymphocytes in the spleen although the absolute number of lymphocytes was normal.

In *infective hepatitis* spleen puncture reveals the presence of many of the immature precursors (some being in mitoses) of those lymphatic plasma cells that are found in the blood. These are the same as the elements we have called lymphatic plasmoblasts which are found in gland puncture in *rubella*. From these findings together with absence of any excess of plasmacytoid reticulum cells in the marrow (in the early stages of infective hepatitis) we infer that the majority of the plasma cells in the blood in this disease are derived from the spleen.

An unusual example of a *lymphatic reaction* was seen in a case of pituitary tumour in which the lymphocytes rose to 18 000 and the spleen became enlarged. Spleen puncture and the subsequent course of the illness excluded the possibility of lymphatic leukæmia and we incline to the view that the lymphatic reaction was of central origin but a coincidence with a *lymphocytosis acuta* cannot be excluded.

while the number of lymphocytes in the peripheral blood decreases and it is only after prolonged administration (or in unduly susceptible persons) that the granulocytes fall (Moeschlin and Meili 1947). In animal experiments the lymphocytes are far more easily reduced than are the granulocytes except in cats (Moeschlin and Naef 1948; Moeschlin and Bodmer 1949). *The action of urethane in the leukemias in the therapeutic employed dose is thus a more or less selective damage to the mitotic activity of the dedifferentiated (neoplastic) white cells.* The splenogram and the myelogram of the white myeloid series shift towards maturity although more slowly while the erythroblasts increase.

In lymphatic leucoses the percentage of lymphocytes in the spleen and marrow shows a reduction although not as a rule a very great one.

Urethane thus resembles arsenic in its power of inhibiting mitosis.

Treatment of a case of chronic myeloid leukemia with *nitrogen mustard* was also followed by decrease of immature cells. Here also there appears to be toxic interference with mitosis in addition to direct destruction of immature cells. Similar changes can be observed after *radio active phosphorus*. Both these substances have therefore effects very similar to those of λ rays.

Sections of spleen puncture material in the early stage of a case of *paramyeloblastic leucosis* showed that initially the abnormal cells have a focal arrangement similar to that of sarcomatous metastases. Only later is the organ diffusely infiltrated.

Spleen punctures from 3 cases of *erythroleukemia* are described.

In a detailed consideration of the *primary malignant reticuloses (reticulo endothelioses)* an attempt based on spleen marrow and gland punctures is made to clarify this condition and to separate it from other diseases to which the name has been improperly applied. Special attention is devoted to cases in which only the spleen and glands were infiltrated by the tumour cells which were also present in the blood while the marrow was free from involvement.

The characteristic cells are described in detail and the points of distinction between them and other tumour cells are stressed.

The disease is regarded as being neoplastic at first being localised but later metastasising so as to present the apparently systematic distribution which has misled pathologists who see only the terminal phase.

The relationships of reticulosis lymphadenosis and undifferentiated sarcomas is considered.

Special attention has been given to *the changes in the blood proteins* that often occur in such cases and much emphasis is laid on the function of the reticulo endothelium in relation to the formation and transformation of protein substances. There is an interesting

the myeloblasts were between 2.5 and 17 per cent, died within 9 months

Comparative examinations of spleen and marrow films show that the cellular picture is more immature in the latter, and we suggest that this may be due to splenogenic (endocrine) inhibition of the myeloid tissue. On the other hand, this view seems to be contradicted by the fact that there were always 3 or 4 times more mitoses in the granulocytes in the marrow than in those in the spleen. The mitoses were almost confined to the immature and half mature myelocytes, and it may be that the high incidence of mitoses in the marrow is simply the result of its high content of undifferentiated cells.

Spleen punctures allow the effect of treatment in chronic myeloid leukaemia to be followed especially when the marrow is also examined. *X irradiation is followed by great reduction and maturation of the myeloid tissue in the spleen whereas typical leukaemic changes persist in the marrow. Similarly the mitotic index in the spleen falls sharply but remains unchanged in the marrow. It can therefore be said that the effects of X irradiation in chronic myeloid leukaemia remain localised, unirradiated myeloid tissue being unaffected. The fact that irradiation of the spleen is followed by almost complete return to normal of the hæmogram (in spite of the persistence of leukaemic changes in the marrow) seems to be clear proof that the abnormal cells so characteristic of the blood picture in chronic myelocytic leucoses, are derived from the spleen (and perhaps the liver). A view first propounded by Rohr.*

Our view is that chronic myelocytic leukaemia should not at least in the early stages be treated by general irradiation or by irradiation of the bones, because irreparable damage may be done. Careful treatment over the spleen will usually suffice.

The effects of arsenic have also been investigated by spleen and marrow punctures. In both tissues arsenic produced a decrease in the percentage of granulocytes (mainly of the myelocytes but also of more mature forms) together with a relative increase of erythroblasts. Arsenic also leads to diminished mitotic activity in both the spleen and the marrow. In one case in which arsenic had been given in small doses for 6 years the number of granulocyte mitoses was reduced to a fortieth.

We agree with Forkner that treatment with arsenic is to be preferred to radio therapy especially because it increases the expectation of life. Its effect depends mainly upon toxic inhibition of mitosis as has also been shown by Dustin.

In cases of chronic leukaemia treated with urethane there was great reduction in the number of mitoses in the abnormal cells in the spleen but the mitotic index of the erythroblasts rises. In normal persons urethane does not usually inhibit mitotic activity.

while the number of lymphocytes in the peripheral blood decreases and it is only after prolonged administration (or in unduly susceptible persons) that the granulocytes fall (Moeschlin and Meili 1947). In animal experiments the lymphocytes are far more easily reduced than are the granulocytes except in cats (Moeschlin and Naef 1948; Moeschlin and Bodmer 1949). *The action of urethane in the leukæmias in the therapeutic employed dose is thus a more or less selective damage to the mitotic activity of the dedifferentiated (neoplastic) white cells.* The splenogram and the myelogram of the white myeloid series shift towards maturity although more slowly while the erythroblasts increase.

In *lymphatic leucoses* the percentage of lymphocytes in the spleen and marrow shows a reduction although not as a rule a very great one.

Urethane thus resembles arsenic in its power of inhibiting mitosis.

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parallelism between reticuloses and plasmocytic myeloma, which also belongs in this group

Our experience shows that changes in the blood proteins do not generally occur in association with those reticuloses in which there is plentiful formation of fibrils, presumably because the protein forming power of the cells is diverted to fibril formation. Changes in the proteins (high B S R positive cephalin and cadmium reactions, electrophoretic changes, and abnormal globulins) are more frequently seen in cases in which the fibril formation is absent—presumably the unfixed proteins make their way into the blood.

The structure of various kinds of *sarcoma cells* and the possibility of finding *carcinoma cells* (if a mass erroneously supposed to be an enlarged spleen is punctured) is described. The occurrence of sarcoma and carcinoma cells in groups unlike the isolated cells of lymphosarcoma and reticulosis is regarded as a valuable differential feature.

The first observations of lymphosarcoma lymphoblastoma and other sarcomatous cells as seen by *phase contrast microscopy* are here recorded and the method advocated as a valuable aid for the differential diagnosis of tumour cells.

Finally some *rare causes of splenic enlargement* are described viz *Gaucher's disease*, *Niemann Pick disease* and some rather obscure cases of splenomegaly associated with lipæmia.

Based on the present examinations the spleen puncture is found to be a harmless and valuable clinical method of examination taken that the proper described technique is used. Our observations have led us to regard spleen puncture especially if combined with marrow puncture as being of great clinical value in the hæmatological and diagnostic sense.

EPILOGUE

This completes our discussion about spleen puncture. We hope that the communicated results have clearly proven the clinical value of this simple method of examination which if properly handled and indicated is quite safe. May it take its due place in the clinical examination technique. Like all hæmatological and cytological methods spleen puncture requires a certain experience and its use in clinical diagnosis should be in relation to the whole of the clinical picture in each case. On this basis spleen puncture is in many cases especially where a simultaneous sternal puncture is made a valuable and safe supplement of the clinical methods of examination of the present day.

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INDEX

- Adrenalin test 7
- Agranulocytosis
 - cyclical 99
 - due to urethane 152 158
 - in infective mononucleosis 83
- Aleukemic leucosis 53 125
- Amiotosis 41 43
- Amyloidosis 198
- Anæmia
 - aplastic 58 65 73
 - extra medullary blood formation 71
 - hemolytic 51 63
 - osteosclerotic 60 61 64
 - pernicious, 73
- Antibody formation of plasma cells 14
 - 3 34 98
- Arsenic treatment
 - changes in spleen and marrow due to 145
 - effect on mitoses 147
- Bang's disease 111
- Banti's syndrome 55
- Basophiles *See* Tissue mast cells
- Blastomycosis 112
- Blood formation extra medullary in
 - spleen 16 34 60 80 168 169
 - chronic inflammatory 81 94 108
 - erythroblastic 63 108 135 172
 - compensatory 60 64-69
 - leukæmic 130
 - myelocytic 34 61 64 130
 - megakaryocytic 57 63 79 134 16
- Boeck's disease 51 109
- Brill Simmers disease 191
- Carcinoma cells 197
- Carcinomatosis of bone marrow 61
- Cells
 - differentiation 11 44
 - division
 - amitotic 41 43
 - mitotic 37
 - epithelioid 103 110 111
 - genesis 12
 - immature 34
 - lymphatic 30
 - morphology 17
 - myeloid 34 61 64 130
 - pathological 37
 - reticulo endothelial 17
- Cholangitis 8.
- Cirrhosis 53
 - plenomegalic 51 55
- Coloration technique of 10
- Complications of spleen puncture 9
- Culture of bacteria from spleen puncture 80 95
- Cyclical agranulocytosis 99
- Cysts 197
- Endothelial cells 17
- Endothelium capillary 18
- Eosinophilia 58 100
 - persistent 01
- Epithelioid cells
 - in Boeck 110
 - in brucellosis 111
 - syphilitic 110
 - tuberculous 103
- Erythroblastophthisis 70 73
- Erythroblastoses 172
- Erythroblasts, 16 34 60 63 86 108 134 172
 - mitoses 39 175
- Erythro-leukosis 173
- Febris undilans 111
- Felty's syndrome 51 99
- Film technique 8
- Fixation methods of 10
- Gaucher's disease 198
- Germ centre cells 21 30 *See also*
 - Reticulum cell large lymphatic
- Giant cells
 - blastomycotic 11.
 - Sternberg 113
 - syphilitic 110
 - tuberculous 104
- Glandular fever 82
- Globulins β and γ 14 127 177
- Glycogen storage disease 01
- Granulisation toxic 81 96
- Granulocytes 35
- Granulomatosis 103
- Hæmoblastoses 119
- Hæmochromatosis 58 01
- Hæmocytoblast 12
- Hæmohistoblast 12
- Hæmolytic anæmia 51 63 112
- Hepatitis epidemica 88
- Histological technique 10
- Histoplasmosis 103
- Historical 1
- Hodgkin's disease 51 113
- Hypernephroma 197
- Hypersplenism 50 55 76 140 186
- Kala azar 51 10
- Karyokinesis 37
- Langhans giant cell 104
- Liver cirrhosis 53
 - splenomegalic 55
 - inflammatory 6
- Leishmaniasis 102

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- Polycythæmia vera 171
- Protein formation
by lymphocytes 42
by plasma cells 14 34 99 180
by reticulo-endothelium 177
- Pulp cells 19 90 96
hæmosiderin phagocytosis 69
mitosis 37
- Puncture *See* Spleen puncture
- Q fever 90
- Radio-active phosphorus 166
- Reaction
lymphatic 82
neutrophile III
possibly of central origin 91
- Reti culum cells
large lymphatic A 30
lymphocytoid 0
mitoses 37-39
phagocytosis 27 26 77 9
plasmocytoid 12
protein formation 14 177 178
small lymphatic A 89 98
- Reticuloses, 51 147 176
- Retothel sarcoma 195
- Rickettsiasis 90
- Rubella 13 8
- Sarcoidosis (Boeck) *See* Boeck's disease
- Sarcoma 51 189 194 195
lymph 189
melano 194
retothel 195
undifferentiated 195
- Schistosoma 57
- Schuller Christian disease 00
- Sepsis lenta 95
- Serosa cells 18
mitoses 37
- Spleen
palpation 7
X ray demonstration of 3
- Spleen puncture
abscess 9
acute inflammatory 79
chronic inflammatory 93 108
complications 9
contra indications 6
count 11 45
diagnostic significance 48 54 68 102
103 117 119 178 189 198 200
histological investigation 5 10
historical 1
- Spleen puncture—*contd*
instruments for 5
lymphatic reaction 82
negative 9
neoplastic diseases 119
neutrophilic reaction 80
site of 3
technique of 6
- Splenectomy 57 68 97 109 178 140
- Splenogram 44
errors 44 45 171
variations 48
- Splénomegaly
inflammatory 79
blood formation 64
Non inflammatory 53
with lymphocytosis possible central
91
uncertain pathogenesis A 01
- Staining 10
- Sternal puncture
differences from spleen puncture
in leukæmias 135 138 144 154
in reticulosis 183 189
- Sternberg cells 113
- Still Chauffard Felty disease 97
- Storage diseases 51 198
- Syphilis 51 110
- Technique
of films 8
of puncture 6
- Thrombocytes 35 76
- Thrombocytopenia 64
in hæmolytic anæmia 76
in infectious mononucleosis 83
with splénomegaly 75
toxic 77
- Thrombosis of splenic vein 51 58
- Tissue mast cells 27 86 97 125
- Tuberculosis 103
acute hæmatogenous 80 106
chronic of spleen 51 107
miliary 107
- Turk cells 12
- Urethane 150 08
- Virus diseases with splénomegaly 82 III
90 91
- X rays
effects on mitoses 142
effects on marrow in leukoses, 140
effects on lymphosarcoma 191

- Leucoses** 119
 aleukæmic 53 125
 basophile 175
 changes in spleen and marrow due to
 arsenic 145
 urethane 150
 X rays 140
 Differences of cellular composition in
 spleen and marrow 135
 early 131
 eosinophile 175
 erythro 172
 lymphatic 120 125 127
 megakaryocytic 175
 mitoses number of in spleen and
 marrow 138
 myelocytic 130
 paramyeloblastic 167
 reticulocytic 176
Lipæmia 201
Lymphadenosis 120
 acute 127
 aleukæmic 125
 chronic 120
 isolated of the spleen 125
 sub leukæmic 126
Lymphatic reaction 82
 of possible central origin 91
 in virus diseases with splenomegaly 90
Lymphatic cell series 30
Lymph follicles in the spleen 11 16 44
Lymph gland puncture 13 18 33 87
 104 110 111 113 182 185 193
Lymphoblasts 31 41 123 129
 mitoses 39 42
**Lymphoblastoma folliculare (Brill
 Simmons)** 191
Lymphocytes
 function 14 42
 life span 41
 production 41
Lymphocytosis
 of central origin 91
 acute 91
Lymphogranulomatosis 113
Lymphosarcoma 51 127 189

Macroblasts 34 72
Macrophages 22
 bacterial 25
 cell debris 22
 erythrocytes 71
 fat 25
 lipoid 25
 hemosiderin 23
 large monocytoïd 25
 melanin in malaria 24
 parasite 25
 pigment 22
Malaria 51 100
Marking of cells in films 31
Mast cells histiocytic 27 86 97 125
Megaloblasts 74
Megakaryocytes
 leukoses 175
 occurrence 34 57 63 73 78 134 176
Melano-sarcoma 194
Mitoses 37 40 42
 effect of arsenic 145
 Mitoses—*contd*
 effect of X rays 142
 in erythropoietic series 39
 in mononucleosis 85
 haploid 169 170
 index 138
 lymphatic series 39 40
 megaloblasts 74
 myeloid series 40
Monoblasts lymphoid 85
 mitoses 85
Monocytes 12
 histiocytic 12 27 35 96
 lymphatic 12 35
 myeloid 12 35
 origin 12 27
Mononucleosis infectiosa 82
Morbus Bang 111
 Banti 55
 Boeck 109
 Chauffard 97
 Felty 97
 Gaucher 198
 Niemann Pick 200
 Schuller Christian 200
 Still Chauffard Felty 97
 Werthof 76
 Wilson 58
Myeloblasts
 mitoses 40 169
 occurrence 35 66 169
 reaction in chronic leucosis 131
Myelocytes
 mitoses 40
 occurrence 34 60 79 82 86 90 130
 138 142 148 155
Myeloma 14 32 (Fig 20e)
Myelosis . See Leucoses

Niemann Pick disease 9
Nitrogen mustard treatment
 in leukoses 160
 in lymphoblastoma 193
 in lymphosarcoma 191

Osteosclerosis and osteomyelosclerosis 64

 Pernicious anemia 73
Pfeiffer's disease 82
Pituitary tumour 91 203
Phagocytes . See Macrophages
Phagocytosis
 bacteria 25
 erythrocytes 71
 in plasmocyt retic cells 15 29
Phase contrast 32 74 84 191
Phosphorus radio-active 166
Pigments
 ferruginous 22
 iron free 22
Plasmoblasts 32
 mitoses 39
Plasma cells
 hemosiderin deposits in 29
 lymphatic (differences from reticular)
 13 29
 mitoses 39 40
 origin 17
Polyarthrits 63

